

formic acid product and for poly-O-acetyl-DL-serine, but not for the material obtained after poly-DL-serine underwent treatment with concentrated sulfuric acid. These tests are suggestive of the absence of peptide-bond alterations for the formic acid treated materials and for elimination of all peptide bonds in the sulfuric acid treated materials.

The presented results as well as the additional evidence obtained by the use of model compounds indicate that formic acid under the conditions used is undoubtedly an excellent formylating agent. Recently Narita⁹ and Neurath¹⁰ have reported similar conclusions on the use of formic acid on various proteins. A more extensive article is in preparation for *Compt. Rend. trav. lab. Carlsberg*.

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Effect of Lysine on the Purification of Human Plasminogen on Cellulose Ion Exchangers

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Plasminogen, the precursor of the proteolytic enzyme plasmin, is present in mammalian blood plasma. Experience has shown that it is rather difficult to purify it. One of the reasons seems to be that the

plasminogen has a marked tendency to coprecipitate with other proteins (Remmert and Cohen¹). The purest plasminogen preparations have been prepared according to the method of Kline². This method is based on the resistance of plasminogen to acid and alkali denaturation. In the present work an alternative method is outlined.

According to Müllertz and Lassen^{3,4} there are in human plasma two different factors concerned in the fibrinolytic system when streptokinase is used as activator, the plasminogen and the proactivator. After activation with streptokinase the former gets proteolytic and the latter plasminogen activating activity. Test methods, in which bovine fibrin containing plasminogen is used as substrate measure mainly the activator activity whereas those methods in which casein or another protein is used as substrate measure the proteolytic activity.

The preparations and fractions obtained in this work have been tested with the caseinolytic method of Müllertz⁴ and the fibrinolytic method of Christensen⁵. As no significant separation between the two plasma factors has been obtained and because the caseinolytic method seems to be the more reliable one of the two methods, the figures in the text concerning yield and specific activity refer to this method.

Attempts have been made to reproduce the method of Kline. The proteolytic potency of the preparations obtained has varied considerably. The activity per mg substance has been 5 to 10 times that of Fraction III (Cohn) as prepared by Oncley *et al.*⁶ According to our calculations this should mean an increase in activity of about 100 to 200 times as compared to plasma on a protein basis, which is 2 to 4 times lower than the result published by Kline. The yields have been low, only 5 to 15 % from Fraction III.

In order to avoid this rough handling and the accompanying low yields we have tried other, milder forms of separation. A partial purification of plasminogen has been obtained by dialysing a suspension of Fraction III paste against a 0.02 M sodium phosphate buffer of pH 7.0 during 20 h at +5°C. A precipitate is obtained, which contains 12 to 15 % of the protein in the starting material. The precipitate has a specific caseinolytic and fibrinolytic activity which is 4 to 5 times that of Fraction III. The yield of plasminogen is about

60%. The precipitate is very slightly soluble in buffers of neutral reaction.

The preparations were purified chromatographically on DEAE-cellulose of the Sober and Peterson type⁷. They were dissolved in 0.02 M ammonium acetate buffer pH 9.0 and applied to DEAE-cellulose columns previously equilibrated with the same buffer. Under these conditions most of the protein as well as the plasminogen is adsorbed on the DEAE-cellulose. The elution was performed stepwise with buffers of increasing acidity, from pH 9.0 to pH 4.0. Plasminogen appeared in several of the fractions but no or very slight increase in specific activity was obtained. The yields were very low.

Recently Alkjaersig, Fletcher and Sherry⁸ reported that ϵ -aminocaproic acid, lysine and certain other basic amino acids in low concentrations increased the solubility of plasminogen and plasmin at neutral pH. This effect of lysine may depend on a specific interaction between plasminogen and lysine. To see if an addition of lysine to the buffers used for elution would give a better separation of plasminogen at the chromatography on DEAE-cellulose the following experiment was performed.

About 5 g of DEAE-cellulose (Brown Comp. New Hampshire, USA) were suspended in 0.02 M ammonium acetate buffer, pH 8.9 to 9.0, containing lysine to a concentration of 0.01 M. The slurry was poured in a column with a diameter of 2 cm and allowed to settle under a slight hydrostatic pressure. The column was equilibrated with the same buffer. 125 mg of the partially purified plasminogen preparation obtained through dialysis against phosphate buffer were dissolved in 12.5 ml of the equilibration buffer at pH 8.9. Some of the material did not dissolve and was removed by centrifugation. It contained about 10% of the protein and 3% of the plasminogen activity. The opalescent supernatant was applied to the column, which was then developed with about 200 ml of the equilibration buffer. The elution was performed stepwise with the following buffers: 0.02 M sodium phosphate pH 6.5, 0.02 M sodium citrate pH 5.5, 5.0 and 4.0. All buffers contained lysine to a concentration of 0.01 M. The effluent was collected in fractions of 10 ml/h. The protein content of each fraction was estimated with the color reaction of Herriott-Lowry^{9,10}. The fractions corresponding to each peak in the chromatogram were pooled, lyophilized and redissolved in 0.15 M sodium phosphate buffer pH 7.3. After dialysis against the same

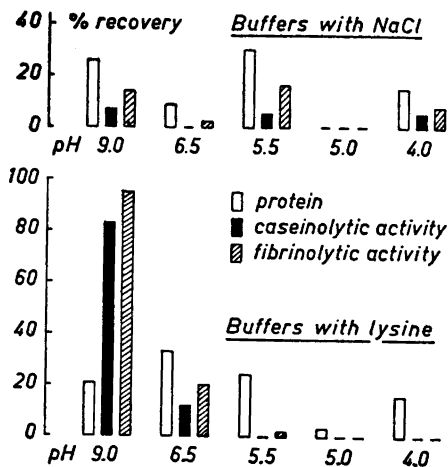


Fig. 1. Chromatography of partially purified plasminogen on DEAE-cellulose. The diagrams show the distribution of protein, caseinolytic and fibrinolytic activity among the fractions that appear at different pH. Upper diagram shows the pattern obtained when 0.01 M NaCl is added to the buffers. Lower diagram shows the pattern obtained when 0.01 M lysine is added to the buffers.

phosphate buffer the volumes were made up to 50 ml and the fractions analysed in respect to protein concentration, caseinolytic and fibrinolytic activity. For comparison another chromatogram was made at the same time and in exactly the same way but with the exception that lysine was replaced by sodium chloride. The results are shown in Fig. 1.

The upper part of Fig. 1 is an example of the pattern obtained in the chromatography without lysine. About 10% of the plasminogen appears with the front. In the subsequent eluates the activity appears in the fractions containing most protein (pH 5.5 and 4.0). The yield and the specific activity in these fractions is low. The total yield of plasminogen is 20 to 25%.

If lysine is added to the buffers the behaviour of plasminogen is markedly changed. The main part of the caseinolytic and fibrinolytic activity is not adsorbed to the DEAE-cellulose but appears in the front. The remaining part of the activity appears when elution is performed with 0.02 M phosphate buffer pH 6.5. In the frontal

peak about 20 % of the protein of the starting material appears together with about 80 % of the plasminogen. Consequently the starting material has been purified 4 times. This means an increase in purity of 16- to 20-fold as compared to Fraction III and the yield from Fraction III is about 50 %.

The reason for this behaviour of plasminogen is not understood. Attempts to adsorb the plasminogen to CM-cellulose equilibrated with buffers of pH 8.9 containing lysine have not been successful, which indicates that there is no reversal in the electric charge of the plasminogen.

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Separation of Glycerol Ethers by Gas-liquid Chromatography

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In the course of studies^{1,2} on the metabolism of the 1-mono-ethers of glycerol with hexadecyl, octadecyl and 9-octadecenyl alcohol, *i.e.* chimyl, batyl and selachyl alcohol, a method for the separation of the individual glycerol ethers was needed. This separation has also earlier been made with adsorption chromatography on

alumina³, but any separation of batyl from selachyl alcohol was not obtained.

The separation of methyl esters of high molecular weight fatty acids by gas-liquid chromatography (GLC) has been reported⁴⁻⁶. Since GLC permits microgram mixtures of methyl esters of fatty acids to be resolved, analysis of mixtures of glycerol ethers has been investigated.

The present paper describes the complete separation of chimyl, batyl and selachyl alcohol by GLC after acylation of the two hydroxyl groups of the glycerol ethers.

Apparatus and procedure. The GLC experiments were carried out with an Argon Pye Chromatograph. The temperature of operation was 218°C and the inlet pressure of the gas 72 cm Hg. The rate of flow of the carrier gas (Argon) was 20 cm³/min. The detector voltage was set on 1250 V. Each 4-foot column was packed with 5 g of acid-washed, alkali-treated Celite (mesh 100-140). As stationary phase a polar polyester LHC-1R-296 (obtained from Cambridge Inc., Mass. U.S.A.) was used in the ratio Celite: stationary phase 4:1. Acylation of the two hydroxyl groups of the glycerol ethers was carried out with acetic anhydride in the presence of pyridine after boiling on a water bath for 12 h. The esterified mixture of glycerol ethers was isolated and dissolved in a few microlitres of ethyl acetate, and the solution taken up in a micropipette and applied to the column.

The retention volumes of the different glycerol ethers were determined using a mixture of known compounds (Table 1). The retention volumes are expressed relative to synthetic batyl alcohol, which was used as internal standard. When determining the retention volumes of the pure compounds, special care was taken not to overcharge the column in order to obtain symmetrical peaks on the chromatogram. The polyester column had a retention time of batyl alcohol diacetin of 49 min.

Table 1. GLC characteristics on LHC-1R-296 as stationary phase of three glycerol ethers after acylation. Temperature 218°C. Argon flow rate 20 cm³/min.

	Apparent retention volume relative to batyl alcohol- diacetin = 1.00
Chimyl alcohol	0.61
Batyl alcohol	1.00
Selachyl alcohol	1.11