Short Communications

Studies on the O,N-Acyl Shift

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Elliott¹, Lucas², and others demonstrated the occurrence of an O,N-acyl shift³ in various proteins and model peptides⁴ under the influence of concentrated sulfuric acid, anhydrous phosphoric acid and other strong reagents. Josefsson and Edman⁵ reported the reversible inactivation of lysozyme and ribonuclease due to an O,N-acyl shift induced by the mild action of "100 % formic acid" at 22° for 24 h. This preliminary note presents the results of studies of the action of anhydrous formic acid on various model compounds.

The infrared spectra of the above compounds were determined by the use of the Scheidt method⁶. The infrared spectra were also determined on the recovered materials after treatment with anhydrous formic acid and with concentrated sulfuric acid. The differences between the spectra of the original compounds and those of the formic acid treated peptides were not extensive, whereas the spectra of materials recovered after sulfuric acid treatment differed greatly from both those of the formic acid treated material and the original. Due to its waxy appearance, however, the sulfuric acid treated material gave poor infrared spectra by the Scheidt technique.

Table I shows the results obtained from the incubation of ¹⁴C-formic acid with various substrates at 22° for 24 h.

Contrary to the findings of Josefsson and Edman⁵ these results clearly indicate

Table I. Radioactivity recovered in c.p.m. per mg carbon.

		Ext 1	Ext 2
1	Anhydrous ¹⁴ C—formic acid used	6 050	68 000
2	Poly-DL-serine after treatment	1 095	19 500
3	The above (2) after repeated washings	1 165	19 400
4	The recovered formate hydrolysis of the washed material (3)	5 310	63 000
5	The recovered poly-DL-serine after hydrolysis of the washed material (3)	4	11
6	Poly-DL-alanine after ¹⁴ C-formic acid treatment	4	—

that formylation occurred in the serine polypeptide during the action of the formic acid, yet this data do not show if an O,N-acyl shift occurred.

Poly-DL-serine incubated at 22° for 24 h with anhydrous acetic acid produced a compound that by infrared spectra and independent synthesis⁷ was found to be poly-O-acetyl-DL-serine. Poly-DL-serine is exceedingly water soluble and deliquescent; both the prepared poly-O-acetyl-DL-serine, as well as the recovered product after treatment with formic acid, were water insoluble. The Biuret test⁸ was found to be positive for poly-DL-serine as well as for its

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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