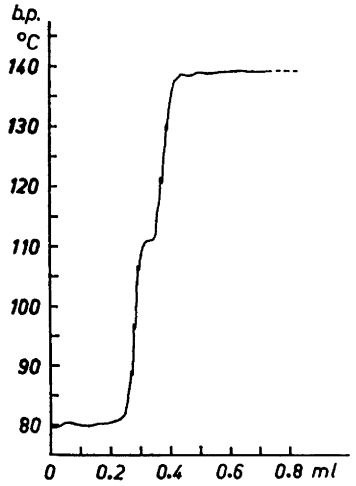

Fig. 3. Distillation curve for a mixture consisting of 0.3 ml benzene, 0.05 ml toluene and 1.0 ml xylene. The temperature was recorded by means of a thermocouple and a recorder.

This will prevent the formation of large drops.

Fig. 3 shows a distillation curve obtained with the apparatus shown in Fig. 2.

It may even be said that this kind of outlet can be applied to larger distillation apparatus. In that case, the system will be designed in such a way that the condensate will drop on the end of the capillary which will have the form of a small cup, with a "dropping tip" at the "outer" bottom.

The construction of the apparatus was executed by the glassblower's firm of Werner Glas (Stockholm).

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N-Terminal Amino Acids of Human and Bovine Prothrombin

STAFFAN MAGNUSSON

Chemistry Department II, Karolinska Institutet, Stockholm 60, Sweden

Miller and van Vunakis [1], analyzing bovine prothrombin (~1 500 NIH u/mg), prepared according to Seegers [2], found no N-terminal or C-terminal amino acids, using Sanger's 1-fluoro-2,4-dinitrobenzene method and digestion with carboxypeptidase, respectively.

Recently Miller [3] analyzed bovine prothrombin which he had further purified (~2 000 NIH u/mg) by chromatography on the carboxylic cation exchanger Amberlite IRC 50. He found one mole of N-terminal alanine per mole of prothrombin, again using Sanger's method.

The present author has analyzed bovine and human prothrombin, both prepared according to Seegers [2], using the phenyl-*iso*thiocyanate method of Edman [4] for determination of N-terminal amino acids.

Experimental. Bovine prothrombin was prepared from 20 liter portions of bovine blood. The yield of prothrombin, when freeze-drying the isoelectric precipitate at pH 4.6, after first discarding the precipitate at pH 5.4, was generally 300—400 mg.

Human prothrombin was prepared by the same method from one lot of 3 200 ml pooled plasma from some fifty donors. The yield was 116 mg.

Two methods were used for routine determination of activity.

1. The prothrombin-proconvertin method of Owren and Aas [5], with the modification that barium sulfate adsorbed bovine plasma according to Brodthagen [6] was used as source of fibrinogen and factor V.

2. Activation of prothrombin by dissolving it in 25 % w/w of aqueous trisodium citrate according to Seegers [7]. Clotting activity of the thrombin generated was determined every 15 min, using as substrate bovine fibrinogen (Blombäck and Blombäck [8]) in tris(hydroxymethyl)amino methane buffer of ionic strength 0.15 and pH 7.5.

One of our bovine preparations was compared with a sample of prothrombin (No. 55/02/10) containing 26 000 U/mg tyrosine, obtained from Dr. Seegers and was found to have the same activity as the one of

Seegers, when activated in 25 % sodium citrate. All preparations used in this work had the same or slightly higher activity.

The general procedure for determining N-terminal amino acids was the following. To 0.3 μmoles of prothrombin dissolved in 1 ml of water and 2 ml of pyridine is added 0.1 ml of phenyl*iso*thiocyanate. Coupling is performed at room temperature, keeping the pH at 8.4—9.0 by occasionally adding 0.1 M NaOH. Excess phenyl*iso*thiocyanate is then removed by washing once with benzene and repeatedly with acetone. (In quantitative determinations, where amino acids are added, the acetone washing is replaced by several washings with benzene and freeze-drying.) The vacuum-dried PTC-derivative is then heated in 1 M HCl on a boiling water bath for 60 min. PTH-Amino acids are extracted with ethyl acetate and identified by paper chromatography, using solvents D and E of Edman and Sjöquist [9] and an heptane:formic acid: ethylene chloride solvent (Sjöquist [10]). Spots are visualized by ultraviolet fluorescence on a cadmium borate screen [9] and eluted over night in 95 % ethanol at room temperature. The technique described is mainly in accordance with Blombäck and Yamashina [11].

Results. In qualitative experiments on bovine prothrombin only alanine was identified as N-terminal amino acid in several different preparations. No positive evidence for the occurrence of histidine or arginine in N-terminal position could be obtained.

Assuming the yield of PTH-alanine from N-terminal alanine in prothrombin to be the same as from added free alanine, we found the following amounts (calculated on moisture-free substance and using the value 62 700 for the molecular weight [12]):

Prothrombin, No.	Moles N-term. alanine per 62 700 g prothrombin	Solvent system
33	0.80	D, E[9]
43	0.87	D, E[9]
67	0.85	Sjöquist [10]

With human prothrombin for lack of material only one determination was made. In this case also alanine was found to be the only N-terminal amino acid. Its PTH-derivative was identified by paper chromatography in solvent D and the above mentioned solvent of Sjöquist [10]. Furthermore it had an absorption spectrum in the ultraviolet which is characteristic for PTH-amino acids. Quantitatively 0.7 moles/mole were found, assuming the human and bovine prothrombins to have the same molecular weight.

Preliminary experiments to determine changes in N-terminal amino acids during activation in citrate were performed on bovine prothrombin. At maximum thrombin clotting activity the activation mixture was found to contain alanine, glycine, *iso*leucine (or leucine), phenylalanine, threonine, aspartic and glutamic acids in N-terminal position.

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