Terminal amino acids of bovine prothrombin and thrombin preparations

The phenomena of blood coagulation are fundamentally concerned with the chemical properties of prothrombin and thrombin. Until recently most of the literature in that field was written without information on the properties of prothrombin and thrombin as molecules. Both have now been obtained in purified form[1,2] and in sufficient quantity for chemical studies, and new frontiers have been opened.

It has been reported[3,4] that purified bovine prothrombin has one mole of alanine as N-terminal amino acid, and we have now also found this in all of our preparations. In thrombin obtained from prothrombin either by activation in conc. aq. sodium citrate or bioactivation the N-terminal amino acid is glutamic acid[5]. We can also confirm the reports that no C-terminal amino acids of prothrombin are liberated with the use of carboxypeptidase A or B[3,4]. In fact this is true for all preparations we have studied.

In further work we studied the C-terminal amino acid composition of prothrombin and thrombin by using the ammonium thyocyanate method of TIBBS[6]. In thrombin the C-terminal amino acid is isoleucine. This conclusion is based on chromatography of the hydantoin with five different solvents, and in addition the amino acid in the separated hydantoin was uncoupled and identified by chromatography in three different solvents. To obtain the free amino acid the hydantoin was placed in 0.25 *N* $Ba(OH)_2$ and refluxed for 48 h. The barium was precipitated with CO_2 and removed. The amino acid was taken up from the dried supernatant solution in acidulated acetone and chromatographed.

In the case of the bovine prothrombin from which the thrombin preparations were made we find that the C-terminal amino acids may not be the same, but depend upon the isolation procedure. Altogether we used three different methods, each of which give homogeneous prothrombin as observed with an analytical ultracentrifuge. In two of these procedures the prothrombin obtained contains the C-terminal amino acids tyrosine and glycine. Accordingly there must be at least two open chains in the molecule. In another there is only serine, but in all of them the N-terminal amino acid is alanine. Thus, the difference is specifically at the C-terminal end(s).

The preparation[1] obtained by adsorption on $Mg(OH)_2$ contains tyrosine and glycine. If this preparation is chromatographed with IRC-50 (XE-64) as described[7], the C-terminal amino acids are also tyrosine and glycine, but if it is chromatographed on DEAE-cellulose by methods to be described later, there is only one C-terminal amino acid and it is serine. Although the latter prothrombin has approximately the same sedimentation coefficient by ultracentrifuge analysis as the other two it must be altered by the chromatography with cellulose. The preparative procedure can thus make a difference in the chemical structure, and it is interesting that the activation characteristics of prothrombin made in this way are also unique. Whereas the other two preparations become thrombin in conc. aq. sodium citrate or aq. protamine sulfate the prothrombin made with DEAE-cellulose does not, even though it converts to thrombin very well in the two-stage analytical reagents.

Perhaps the prothrombin obtained from plasma which is first passed through

Abbreviation: DEAE-, diethylaminoethyl-.

a Seitz filter[8] is altered by the filtration and therefore does not become thrombin in aq. sodium citrate. Conceivably the cellulose in the filter could affect the prothrombin in much the same way as the DEAE-cellulose does. In the activation with sodium citrate or protamine sulfate only prothrombin is needed. When it does not activate under those conditions the basis is the difference in the structure of the prothrombin molecule itself, and has nothing to do with the absence of a hypothetical essential procoagulant.

TABLE I

TERMINAL AMINO ACIDS IN BOVINE PROTHROMBIN AND THROMBIN

Preparation	*N-terminal*	*C-terminal*
Thrombin	Glutamic acid	Isoleucine
Prothrombin	Alanine	Tyrosine
$Mg(OH)_2$ only		Glycine
Prothrombin	Alanine	Tyrosine
$Mg(OH)_2$ and IRC-50		Glycine
Prothrombin	Alanine	Serine
$Mg(OH)_2$ and DEAE-cellulose		

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Studies on a paraffin-utilizing pseudomonad

A strain of pseudomonad isolated from oily mud on the underside of a motor-cycle gear box was found to grow with any one of a variety of paraffin hydrocarbons as sole carbon source. The best growth was obtained using octadecane as carbon source in a medium containing salts, buffered to pH 7, with $(NH_4)_2SO_4$ as nitrogen source.