COMMUNICATIONS TO THE EDITOR

EQUILIBRIA IN THE FIBRINOGEN–FIBRIN CONVERSION[1]

Sir:

In the previous work on the clotting of fibrinogen it has been assumed or at least implied that the reaction is irreversible and proceeds to completion. Some results in this laboratory led us to believe that this reaction can be treated as a reversible process. On the basis of information available at present about the clotting process three fundamental steps have been recognized.[2,3,4,5] We have postulated that these three reactions are reversible, involving the equilibria

$$(1)\quad F \xrightleftharpoons{\text{thrombin}} \Phi + P \qquad K_1 = (\Phi)(P)/(F)$$

$$(2)\quad n\Phi \rightleftharpoons \Phi_n \qquad K_2 = (\Phi_n)/(\Phi)^n$$

$$(3)\quad m\Phi_n \rightleftharpoons \text{Fibrin} \qquad K_3 = (\Phi_n)$$

where F is fibrinogen, Φ is activated fibrinogen,[3,5] P is fibrino-peptide,[4] n is a number between 5 and 15, Φ_n is the intermediate product described by Ferry and Shulman,[2,6] m is a very large number, and the parentheses indicate activities of the given components.[7] It is, of course, recognized that this reaction scheme is further complicated by the adsorption of the fibrino-peptide upon the fibrin clot,[4] by the non-activating fibrinogen–fibrin–thrombin interactions described by Waugh and Livingstone,[5] and by the effects of water and *p*H. It is also very probable that various polymeric intermediate products other than Φ_n may exist.

The following experiments[8] give evidence in support of this reaction scheme.

(1) Fibrin clots were washed for 24–48 hours in a large volume of 0.3 *M* KCl solution with at least ten changes of the wash solution. Such washed clots will not clot a 1% fibrinogen solution over a period of three days. Since thrombin is more strongly occluded on fibrin clots than any other protein studied[9] the fact that these clots were free of thrombin indicates that they were also free of other occluded proteins.

Washed clots were placed in salt solutions at *p*H 6.5, ionic strength 0.3 to 1.0 and were allowed to come to equilibrium at 2, 25.0 and 37.0°. In all cases a small amount of soluble protein material was detected in the supernatant and a very pronounced temperature dependence of this concentration was observed. This, in conjunction with experiment (3), tends to indicate that step 3 is reversible.

(2) Both viscosity[6] and flow birefringence[10] results point to the fact that in hexamethylene glycol-inhibited systems the polymerized intermediate Φ_n dissociates on dilution. This tends to support the reversibility of step 2.

(3) Several samples of bovine fibrinogen have been clotted at room temperature. The clots were removed and the supernatants placed at 37.0° for a period of 24 hours. No further clotting took place. The samples were then cooled to 2° and small but definite clots appeared after two hours. These clots redissolved almost completely, however, after 24 more hours at 37.0° and reformed[11] again at 2°.

An attempt has also been made to shift the equilibrium in reaction 1 to the left by adding fibrino-peptide and thrombin to washed, thrombin-free fibrin clots suspended in buffer solutions and then measuring the concentration of soluble proteins in the supernatant at equilibrium. While the precision in these measurements was not high due to experimental difficulties, nevertheless, in all cases, in solutions containing additional fibrino-peptide more soluble protein material was found than in those with no peptide added. These experiments tend to indicate the reversibility of step 1 and of the reaction as a whole.

Quantitative work is now in progress to characterize the postulated equilibria and to determine their respective thermodynamic parameters.

(1) This work was supported by contract N6-onr-26414 between Cornell University and the Office of Naval Research.

(2) J. D. Ferry, Am. Chem. Soc. Meeting Abstracts, New York, Sept., 1951, p. 9C.

(3) K. Laki, *Arch. Biochem. Biophys.*, **32**, 317 (1951).

(4) L. Lorand, *Nature*, **167**, 992 (1951).

(5) D. F. Waugh and B. J. Livingstone, *J. Phys. Colloid Chem.*, **55**, 1206 (1951).

(6) S. Shulman and J. D. Ferry, *ibid.*, **55**, 135 (1951).

(7) The activity of fibrin is assumed to be unity.

(8) In all of these experiments Laki purified fibrinogen[3] (95% clottable) and Seegers purified thrombin were used. We wish to thank Dr. W. H. Seegers for his generous gift of purified thrombin, and Dr. L. Lorand for sending us the details of his unpublished method for the preparation of the fibrino-peptide. The experimental details will be described elsewhere.

(9) P. R. Morrison, THIS JOURNAL, **69**, 2723 (1947).

(10) H. A. Scheraga and J. K. Backus, *ibid.*, in press.

(11) Formation and dissolution of these clots cannot be due to the precipitation of "cold insoluble globulin" since *purified* fibrinogen solutions stored at 2° under similar conditions gave no precipitate.

DEPARTMENT OF CHEMISTRY
CORNELL UNIVERSITY
ITHACA, NEW YORK

M. LASKOWSKI, JR.
D. H. RAKOWITZ
H. A. SCHERAGA

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COMPONENTS OF PODOPHYLLIN. VI. ISOLATION OF TWO NEW COMPOUNDS FROM *PODOPHYLLUM EMODI* WALL

Sir:

In continuation of our search for tumor-necrotizing components of the resin, podophyllin, from different species of *Podophyllum*, we have investigated the resin from *P. emodi* Wall.[1] using chromatography with activated alumina. Three colorless crystalline compounds were isolated. In the order of increasing strength of adsorption these were: podophyllotoxin (36–39% yield),[2] demethylpodophyllotoxin (I) (1.7%), and 1-*O*-(β-D-glucopyran-

(1) We wish to thank Dr. W. G. Bywater of S. B. Penick and Co. for the procurement of the roots from India and the extraction of the resin therefrom.

(2) In addition, a small amount of picropodophyllin (about 0.4%) appeared with the podophyllotoxin. This may have been an artifact.