Unless otherwise stated, the chlorophyll concentrations of the samples of algae and fragment suspensions were of the same order.

Since the PARDEE buffer requires a considerable equilibration period, and the activity of the suspensions declines with time, the mentioned results allow only qualitative deductions. Owing to the ending of the vegetation period of *Spirogyra* the experiments had to be discontinued until next spring. It is hoped to obtain more complete data at that time.

From the present data it may be concluded that fragments of *Spirogyra* chloroplasts are able to show photosynthetic activity with a relatively high rate compared to that in intact algae. The few experiments done by Dr. WILLIAMS suggest that the fragments are also capable of light induced phosphorylation.

Experiments with fragments of *Mougeotia* chloroplasts were unsuccessful. Suspensions of these fragments readily turned brown and did not show any photosynthetic reaction.

A detailed report of this study will appear in a forthcoming issue of this journal.

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Note on the mechanism of activation of human accelerator globulin by thrombin*

The purification of the essential components and cofactors involved in the coagulation of mammalian blood has facilitated studies of the complex mechanisms involved. Recent progress in the isolation and concentration of accelerator globulin (Ac-globulin, proaccelerin, labile factor, Factor V) has aided in establishing the essentiality of this enzyme in the clotting of bovine blood[1]. Accelerator globulin (AcG) appears to play a similar role in the clotting of human blood. However, this has not been thoroughly investigated because of the relative instability and unusual adsorption characteristics[2] of AcG in human plasma.

AcG exists in two forms: a precursor form, plasma AcG, and a potent accelerator form, serum AcG. During the early stages of clotting, the conversion of the precursor to the active form is mediated by minute amounts of thrombin; a mechanism which may contribute to the biological autocatalytic conversion of prothrombin to thrombin. The exact mechanism whereby thrombin elaborates a potent catalyst from the precursor is not known. There are indications that this reaction may involve proteolysis since thrombin clots fibrinogen by splitting off a peptide[3] and it also dissolves the fibrin clot under certain conditions[4]. Thrombin has esterase activity[5] similar to other proteolytic enzymes[6].

In the present communication, we wish to provide evidence that thrombin activates human AcG by a proteolytic mechanism.

Plasma AcG was prepared in relatively purified form from freshly collected human plasma[7] and was assayed by a one-stage procedure[8]. One unit of AcG activity is equivalent to that displayed by 0.01 ml of freshly-collected, citrated human plasma. Human thrombin was prepared from purified human prothrombin[7] by sodium citrate activation[9], and, in some preparations, additional purification was accomplished by zone electrophoresis[10]. The commercial bovine product Thrombin Topical (Parke, Davis and Co., Detroit, Michigan, U.S.A.), was also employed after an initial purification procedure[2]. Thrombin activity was measured by clotting of fibrinogen standardized according to the two-stage method for the assay of prothrombin[11].

In Fig. 1, data are presented for the activation of human AcG by purified human thrombin. Under the conditions of the experiment, one unit of thrombin is capable of eliciting a 10–15 fold increase in AcG activity. The activity reaches a maximum and then decreases somewhat depending on the relative proportion of enzyme and substrate. Similar results were obtained with bovine and human thrombin both of which had been purified by zone electrophoresis.

In Fig. 2 is depicted the increase in 5% trichloroacetic acid (TCA) soluble material following the activation of plasma AcG with thrombin. TCA soluble material was measured at 280 mμ in a

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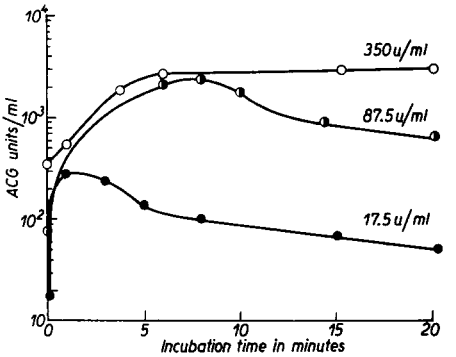

Fig. 1. The activation of varying concentrations of human plasma AcG by thrombin. The AcG having a specific activity of 250 AcG units/mg protein was incubated with human thrombin (1 unit/ml) at 37°C in 0.3% imidazole-0.7% NaCl buffer, pH 7.4. At intervals, aliquots were sampled from the incubation mixture, diluted with saline 1:20 and assayed for AcG by a 1-stage procedure. Values on the right of the figure represent the initial plasma AcG activity. Thrombin alone did not affect the assay system in the concentration used.

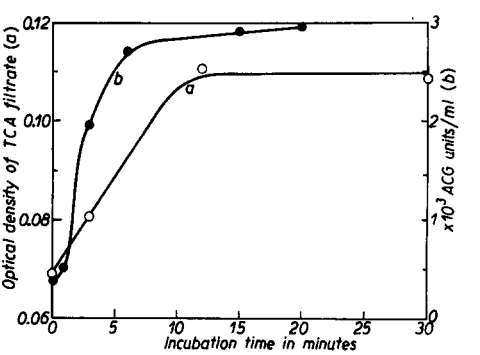

Fig. 2. The relationship between the activation of human plasma AcG with thrombin and the increase in trichloro-acetic acid soluble material. Conditions of the experiment were similar to those listed in Fig. 1. Aliquots of the reaction mixture were taken at intervals, precipitated with an equal volume of 10% TCA and centrifuged at 3,000 *g* for 15 minutes. The optical density of the supernatant was recorded at 280 mμ, pH 0.6.

Beckman DU Spectrophotometer. The material, which failed to precipitate with the addition of TCA, presented absorption throughout the ultraviolet spectrum similar to that of a protein or peptide containing an aromatic amino acid with a peak adsorption at 275–280 mμ.

With heat inactivated plasma AcG preparations (56° C for 30 minutes), thrombin demonstrated no activation or increased elaboration of TCA soluble material. Thrombin did not produce a significant change in TCA soluble material when incubated with human albumin or gamma globulin of comparable protein concentration.

From the above data, it would appear that thrombin acitvates human AcG in a manner similar to that which has been demonstrated in bovine plasma[12].

The question arises whether the activated AcG itself is TCA soluble or insoluble. The evidence obtained so far in our laboratory[13] is not conclusive. However, preparations subjected to less drastic procedures can be isolated chromatographically and are of possible polypeptide dimensions.

In summary, it appears that thrombin has a specific proteolytic effect on plasma AcG which is associated with a significant increase in AcG activity and TCA soluble protein-like material.

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