obtained when $(E_{Zn}-E_{Mg}) \simeq 600$ cal. × mole $^{-1}$. However, the difference is actually 1900 ± 600 cal. × mole, which means that the ratio $P_{Zn}/P_{Mg}$ is about 8:5.

In terms of the transition state theory[10], $P$ is related to the entropy of activation. The standard free energy ($\Delta F^{\ddagger}$), enthalpy ($\Delta H^{\ddagger}$), and entropy ($\Delta S^{\ddagger}$) of activation were calculated from the experimental values of $k$ and $E$. In these calculations the transmission coefficient was assumed to be unity, which usually holds for reactions in solution[10,11], and unit molar concentration was chosen as the standard state. The values of $\Delta F^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ at 22° given in Table I.

TABLE I

KINETIC PARAMETERS FOR THE ENOLASE REACTIONS AT 22° IN PHOSPHATE BUFFER, pH 6.75, $\mu = 0.05$

| *Activator* | $k$ (sec.$^{-1}$) | $E$ (cal. × mole$^{-1}$) | $\Delta F^{\ddagger}$ (cal. × mole$^{-1}$) | $\Delta H^{\ddagger}$ (cal. × mole$^{-1}$) | $\Delta S^{\ddagger}$ (cal. × mole$^{-1}$ × degree$^{-1}$) |
|---|---|---|---|---|---|
| $Mg^{++}$ | 114 | 14600 ± 600 | 14600 | 14000 | − 1.9 |
| $Zn^{++}$ ($Mn^{++}$) | 39 | 16500 ± 200 | 15100 | 15900 | + 2.6 |

To explain the low-temperature inhibition and the different entropies of activation in the reactions catalysed by the $Mg^{++}$ and $Zn^{++}$ enzyme, it is suggested that the enolase molcule can exist in (at least) two distinct forms, *En* and *En'*, which are in equilibrium with each other. In the active complex, the form of the enzyme is *En'* which has a less ordered (more probable) structure than *En*. In the $Mg^{++}$ enzyme, the form *En'* predominates at high temperatures, while the equilibrium is shifted towards *En* at low temperatures, which results in inhibition. On the other hand, $Mn^{++}$ and $Zn^{++}$, due to their greater strength of interaction with the protein, stabilize the more ordered form *En* also at the higher temperatures. Thus, with these ions the formation of the active complex involves a transition from *En* to *En'*, which would explain the positive entropy of activation. The magnitude of the entropy change is too small to involve a major conformation change[12] as suggested by KAVANAU[5]. The structural difference between *En* and *En'*, therefore, probably involves the active site only. This is also indicated by the effect of the nature of the activating ion, whose influence must be restricted to groups at or close to the active site.

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## Photosynthetic activity of fragments of *Spirogyra* chloroplasts

Two types of chloroplasts occur in plants. As a rule higher plants contain grana-bearing chloroplasts while in some algae for instance these plastids are grana-free. In the former chloroplasts lamellar structure is principally restricted to grana embedded in a proteinaceous stroma, but the latter ones are composed of lamellae throughout the whole body and do not contain stroma substance. The platelet-shaped *Mougeotia* chloroplasts and the spirally built *Spirogyra* ones are examples of the lamellate type.

Until recently, attempts to prepare photosynthetically active suspensions of grana-bearing chloroplasts failed. The occurrence in suspensions of quite a number of seemingly intact grana indicates that isolated chloroplasts are readily damaged and also that stromasubstance is much more easily demolished than are grana. The usually observed loss of photosynthetic capacity of isolated chloroplasts, even if they look intact, may be due to changes in the stroma. This suggestion is supported by data of ARNON *et al.*[1,2] who recently succeeded in preparing photosynthetically active chloroplast suspensions when taking special precautions. According to these authors the chloroplasts must be intact; fragments are inactive.

The fact that grana suspensions still show the Hill reaction suggests that a part of the enzyme systems is concentrated in grana while the part responsible for the remaining photosynthetic reactions is located in the stroma.

If this is true the question arises whether in cells with lamellated chloroplasts the latter enzymes occur in the cytoplasm or in the chloroplast lamellae, perhaps acting in cooperation with pyrenoids. If these enzymes occur in the lamellae it may be possible to prepare photosynthetically active suspensions of fragments of these lamellated chloroplasts. It may even be possible to do so without taking the special precautions required for retaining photosynthetic activity of grana-bearing, stroma-rich, chloroplasts.

To test this possibility, suspensions of chloroplast fragments of *Spirogyra* spec. were prepared by grinding the algae at pH 7.2 with sand in a pre-cooled mortar. The suspensions were then centrifuged at 0°C to remove sand and cell debris. The supernatant was used without further washing. Thus no attempt was made to remove cytoplasm remnants, nuclei, and mitochondria. Gas exchange was measured manometrically at 18°C. Oxygen readings were done by using PARDEE buffer in the side bulbs equilibrated with 3% carbon dioxide in air. Carbon dioxide fixation was determined in nitrogen with 5% carbon dioxide, with the side bulbs containing white phosphorus.

We are much indebted to Dr. ANNA MARIA WILLIAMS who, in 3 experiments determined light-induced phosphorylation in aliquots of suspensions used for gas exchange measurements. According to a modification of the method of KING[3] phosphorylation was measured as a disappearance of inorganic phosphate.

The results are shown in Tables I and II.

TABLE I

OXYGEN EVOLUTION AND CARBON DIOXIDE CONSUMPTION OF CHLOROPLAST FRAGMENTS AND INTACT CELLS OF *Spirogyra* IN LIGHT

| *Object* | *Condition* | *Observation period in minutes* | *Number of readings* | *$O^2$ in µl/h* | *$CO^2$ in µl/h* |
|---|---|---|---|---|---|
| Extract | dark | 5 | 31 | −22.4 ±3.0 | |
| | light | 25 | 31 | −2.0 ±1.9 | |
| | light 1st | 15 | 22 | | −22.0 ±4.3 |
| | dark | 15 | 24 | | +2.4 ±2.9 |
| | light 2nd | 15 | 22 | | −8.4 ±1.7 |
| Intact algae | dark | 5 | 14 | −31.2 ±4.5 | |
| | light | 25 | 14 | +16.0 ±6.5 | |
| | light 1st | 15 | 11 | | −13.9 ±9.0* |
| | dark | 15 | 8 | | +1.5 ±5.3* |
| | light 2nd | 15 | 10 | | −3.9 ±3.3* |
| Heated algae | light | 45 | 18 | | −1.0 ±1.3 |
| | dark | 45 | 18 | | −2.4 ±0.8 |

* cell concentration ±1/2

TABLE II

CARBON DIOXIDE UPTAKE AND PHOSPHORYLATION OF FRAGMENTS OF *Spirogyra* CHLOROPLASTS IN LIGHT

| *Expt.* | *Condition* | *$CO_2$ in µl/h* | *$P_i$ in µM/h* |
|---|---|---|---|
| a | light | −33.7 ±7.1 | −2.1 |
| | dark | −2.5 ±6.0 | +0.1 |
| b | light | −12.9 ±7.1 | −0.8 |
| | dark | +6.8 ±8.2 | — |

Out of 18 experiments, 2 yielded negative results. In these two cases, however, even the intact algae did not show photosynthesis. This may be ascribed to the bad condition of the cells due to the end of the vegetation period. One of these two experiments was done in combination with phosphorylation determinations, and no phosphorylation was observed.

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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