EFFECT OF POLYETHYLENE GLYCOL ON HEAT INACTIVATION OF THE HILL REACTION

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SUMMARY

Protection of the Hill reaction from heat inactivation by polyethylene glycol was found in spinach chloroplasts. The protective action of the polymer is discussed in relation to chloroplast membrane structure.

INTRODUCTION

The use of polyethylene glycol in preparing of active chloroplasts from higher plants or algae has been described in many papers[1-8]. In the previous papers[9,10], we confirmed the effectiveness of polyethylene glycol 4000 (PEG) in isolation of photoactive chloroplasts from coniferal leaves. From the data reported so far, PEG seems to have two kinds of actions: 1) elimination of inhibitors released during preparation or storage of chloroplasts; 2) stabilization of chloroplast membrane structure, which is closely related to Hill activity. The present communication deals with the protection from heat inactivation of the Hill reaction by PEG, and its mechanism is discussed.

METHODS

Once-washed chloroplasts were prepared from spinach leaves as described before[11], and suspended in 5 mM Tris-HCl buffer (pH 7.8) containing 40 mM sucrose and 1 mM NaCl. Chlorophyll concentration was determined by the method of Arnon[12]. The chloroplast suspension (0.2 mg chlorophyll per ml) pipetted in a test tube covered with aluminium foil was incubated in a water bath for indicated periods at various

temperatures with shaking in the absence and presence of PEG. After incubation, the suspension was rapidly cooled to 0° C. Hill reaction activity was assayed by measuring oxygen evolution with a Clark-type oxygen electrode at room temperature. The reaction mixture contained the following in μmoles per 3 ml: Tris-HCl (pH 7.8), 50; $K_3Fe(CN)_6$, 3; KCl, 60; $MgCl_2$, 10; chloroplasts equivalent to 0.1 mg of chlorophyll.

RESULTS

Curves 1 and 2 in Fig. 1 show Hill activity-inactivation temperature relation, in the absence and presence of PEG, respectively. Chloroplasts were incubated at various temperatures for 5 min. The incubation without PEG increased Hill activity gradually up to about 40° C (enhancement), and rapidly decreased Hill activity over about 40° C (inactivation), as shown in curve 1. Hill activity was completely lost at about 70° C. The presence of PEG (2 %) gave rise to remarkable resistance against heat inactivation (curve 2), and the inactivation curve shifted towards higher incubation temperatures than curve 1. Curve 3 is the difference between curves 1 and 2 and shows a broad peak at about 47° C. From curve 3, the protective action of PEG was most remarkable in the temperature region of heat inactivation. Furthermore, a slight stimulation of Hill activity was seen in the temperature region of thermal enhancement.

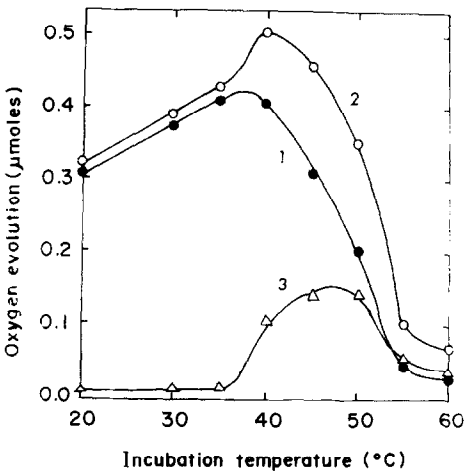

Fig. 1. Dependence of Hill activity on incubation temperature. Hill reaction time, 3 min; light intensity, 80 Klux; curve 1, incubated in the absence of PEG; curve 2, incubated in the presence of PEG (2 %); curve 3, the difference between curves 1 and 2. Other conditions are described in METHODS.

Fig. 2 shows the dependence of Hill activity on incubation time at the incubation temperature of 50° C, which is very close to the temperature with the most effective protection. Curves 1 and 2 show Hill activity-incubation time relation in chloroplasts incubated in the absence and presence of PEG, respectively. It took longer incubation time in the latter case than in the former to obtain an equal degree of inactivation. In both cases, Hill activity initially increased with incubation time and reached a maximum at about 1 min of incubation. Over this incubation time, Hill activity rapidly falled to zero. Curve 3 is the difference between Hill activities in curves 1 and 2 at the same incubation time. The protection by PEG started from the beginning of heat inactivation and covered the whole region of inactivation. The optimum protection was observed at about 2 min of incubation. Hill activity, in this case too, was slightly stimulated by PEG in the region of thermal enhancement.

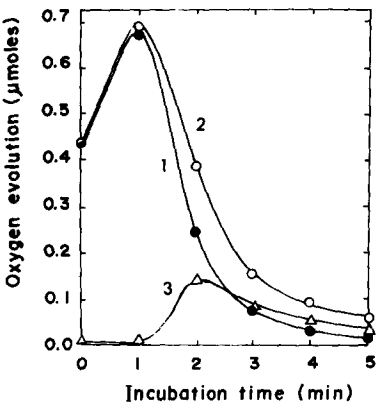

Fig. 2. Dependence of Hill activity on incubation time. Incubation temperature, 50° C; curve 1, incubated in the absence of PEG; curve 2, incubated in the presence of PEG (2 %); curve 3, the difference between curves 1 and 2.
Other conditions are described in METHODS.

DISCUSSION

It is known that heat incubation of isolated chloroplasts leads to the loss of Hill activity but mild heating inversely enhances the activity[1,13]. The thermal enhancement of Hill activity is explained in terms of uncoupling of photophosphorylation with the electron transport system[13].

The enhancememt of Hill activity in the temperature region below about 40° C (Fig. 1) and at the short period of incubation (Fig. 2) is probably caused by this uncoupling mechanism. However, the degree of the enhancement was smaller than that observed on the addition of an uncoupler, NH_4Cl. Therefore, curves in Fig. 1 and 2 can be understood as the superposition of thermal enhancement and inactivation. The former process is predominant on low-temperature or short-period incubation, but is overcome by the latter on high-temperature or long-period incubation.

Heat treatment may bring about various irreversible changes in chloroplasts; structural modofication of chloroplast membrane, liberation of some substances, denaturation of enzymes or proteins, etc.. These changes may be related to thermal inactivation of photosynthetic processes. However, when PEG is present, they are highly suppressed(Fig. 1 and 2).

McClendon[2] reported advantage of use of PEG in preparing of chloroplasts from higher plants or algae. He found that the polymer stabilized chloroplasts and stimulated the rate of the Hill reaction. Clendening et al.[1] described that alfalfa chloroplasts prepared in the presence of PEG (30 %) were contaminated with cytoplasmic protein and the protein was effective for the stabilization of chloroplasts. Furthermore, the protection of chloroplasts from inactivation by heating or aging was also explained as an effect of the contaminated protein. Fry[5] supported their conclusion, but did not elucidate the role of the cytoplasmic protein. Spinach chloroplasts used in the present experiments were isolated without addition of PEG, washed with the isolation buffer, and free from cytoplasmic protein in question.

We consider that adsorption of PEG on chloroplast membrane may protect chloroplast membrane structure from external disturbance such as thermal agitation. The stabilization of chloroplast membrane by an adsorptive interaction of PEG with chloroplast membrane seems to be most responsible for the protection from thermal inactivation. However, elimination of inhibitory substances (e.g. lipids) released during heating by binding to PEG is also an important factor which cannot be neglected for the explanation of the observed phenomenon.

Katoh and San Pietro[14] investigated the thermal inhibition

of Hill activity of Euglena chloroplasts, and demonstrated that the inhibition site was in the electron transport chain between photosystem II and water. Yamashita and Butler[15] reached the similar conclusion in heated spinach chloroplasts.

In conclusion, photosystem II is presumed to have an intimate relation with chloroplast membrane structure, and PEG protects the Hill reaction from heat inactivation through the stabilization of chloroplast membrane structure by an adsorptive interaction.

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