version of phenylglyoxal to ω-aminoacetophenone has been observed after incubation at the optimum pH for approx. 3 h at 37°. By varying the amount of enzyme used, and measuring the conversion of substrate which occurs during incubation for times of this order, a linear relation was obtained between the reciprocals of percentage conversion and enzyme concentration. Extrapolation of these results suggested that 75% conversion would occur at an infinitely high enzyme concentration. Values of 56 and 27% conversion were obtained for relative alanine concentrations of 10- and 3.33-fold, respectively. Chromatographic evidence has been obtained for the formation of alanine during incubation of pyruvate with ω-aminoacetophenone. Alanine formation was dependent on the presence of enzyme preparation, and on the concentration of aminoketone. These results suggest that at equimolar alanine and phenylglyoxal concentrations, the equilibrium position would be unfavourable for ω-aminoacetophenone formation.

An examination of the effect of substrate concentrations on phenylglyoxal transaminase activity, gave values of 12.0 and 0.6 mM for the apparent Michaelis constants of alanine and phenylglyoxal, respectively. These results are similar to those obtained for the alanine-dioxovalerate system[1].

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A coupling factor in photophosphorylation

Several reports have recently appeared which described the isolation from mitochondria of factors which were necessary for phosphorylation but not for electron transport in oxidative phosphorylation[1–6]. The experiments reported herewith will demonstrate the isolation of a similar factor from chloroplasts. It is required for photophosphorylation but not for photoreduction.

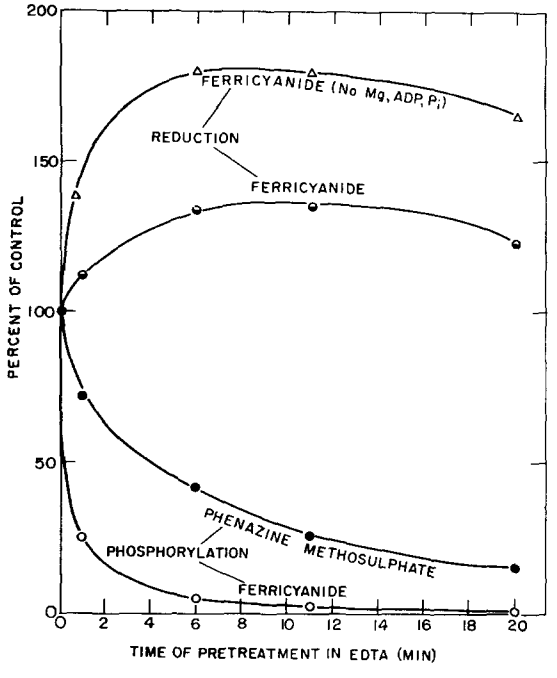

Fig. 1. Uncoupling of chloroplast fragments by pretreatment with EDTA. The reaction mixture contained in a total volume of 3.0 ml in μmoles: Tris, (pH 7.8), 45; NaCl, 60; $MgCl_2$, 12; Na, K phosphate (pH 7.8), 12; ^{32}P, 2×10^6 counts/min; ADP, 4; EDTA (pH 7.8), 1.5; chloroplast fragments containing 35 μg of chlorophyll; and where indicated, phenazine methosulfate, 0.1; and potassium ferricyanide, 1.5. Reaction time, 2 min; light intensity, 160 000 lux; temperature, 20°; chloroplast fragments were prepared as previously described[7], suspended at 0° with EDTA to give a final concentration of 30 μg of chlorophyll per ml and 10^{-3} M EDTA. Samples were removed at the times indicated without any further treatment, for an immediate assay in the various photoreactions. The final concentration of EDTA in the reaction cuvettes was identical in all cases. Control activities in μmoles per mg chlorophyll per h: phenazine methosulfate-dependent photophosphorylation, 370; ferricyanide-dependent photophosphorylation, 74; ferricyanide photoreduction, 275; ferricyanide photoreduction (no Mg, ADP, P_i), 206.

Fig. 1 shows the effect of pretreating chloroplast fragments in EDTA on their ability to perform several photoreactions. It is clear that the pretreatment uncoupled the chloroplasts. It completely inhibited their ability to perform cyclic or non-cyclic photophosphorylation, while stimulating their capacity for photoreduction. In all experiments of this type performed, ferricyanide-dependent phosphorylation was more sensitive than the phenazine methosulphate-dependent one.

Table I illustrates that such chloroplast fragments (pretreated with EDTA following by centrifugation) which lost essentially all their capacity to catalyze photophosphorylation, could be partially reactivated. Reactivation occurred only following the incubation of the pretreated chloroplast fragments in the presence of magnesium ions and the supernatant fraction obtained during the centrifugation of the EDTA-treated chloroplasts. Only 15–40% of the activity of non-EDTA-treated chloroplasts could be restored in different experiments. The restored activity was sensitive to the uncoupling action of ammonium ions[8].

Some properties of the coupling factor (*i.e.*, the factor present in the supernatant),

TABLE I

COMPONENTS REQUIRED FOR REACTIVATION OF EDTA-TREATED CHLOROPLASTS

Reaction conditions as described under Fig. 1, with phenazine methosulfate. Chloroplast fragments containing 16 μg chlorophyll per ml. Chloroplast fragments were suspended at 0° with EDTA to give a final concentration of 30 μg of chlorophyll per ml and 5×10^{-4} M EDTA. The suspension was centrifuged at 20 000 $\times$ *g* for 30 min. The clear supernatant was separated (= supernatant in table), and the pellet was suspended in a small volume of 10^{-4} M EDTA, $1.5 \cdot 10^{4}$ $MgCl_2$ (= EDTA-treated fragments). A similar sample treated in the identical manner but in the absence of EDTA served as a control (= untreated fragments). The components indicated were preincubated for 20 min at 0° before assay. The chloroplast fragments, EDTA and $MgCl_2$ concentration were adjusted to be identical in all the reaction cuvettes. Control activity corresponded to 430 μmoles of ATP formed per mg chlorophyll per min.

Present during preincubation	*ATP formed (%)*
Non-treated fragments	100
Non-treated fragments + supernatant + $MgCl_2$	110
EDTA-treated fragments	2
EDTA-treated fragments + $MgCl_2$ (0.01 M)	8
EDTA-treated fragments + supernatant	3
Supernatant + $MgCl_2$ (0.01 M)	2
EDTA-treated fragments + supernatant + $MgCl_2$ (0.01 M)	35
EDTA-treated fragments + supernatant + $MgCl_2$ + NH_4Cl (3×10^{-3} M)	0

are described in Table II. It was heat labile, non-dialysable and its activity was rapidly lost with storage. The factor was required in a quantitative manner, saturating with excess. Analysis of the protein and nucleic acid content of the supernatant released upon EDTA treatment of washed chloroplast fragments revealed around 0.5 and 0.1 μg per mg chlorophyll, respectively.

The specificity of the coupling factor in restoring the activity of different types of photophosphorylation reactions is described in Table III. Phenazine methosulfate-dependent phosphorylation was in all experiments restored to a higher degree than was ferricyanide-dependent phosphorylation, but both were consistently restored. FMN- or menadione-dependent phosphorylations were not restored to any significant extent in several experiments.

TABLE II

PROPERTIES OF THE COUPLING FACTOR

Reaction conditions as described under Table I, but with 19 μg chlorophyll per ml. Control activity corresponds to 320 μmoles of ATP formed per mg chlorophyll per h.

*Present during preincubation**	*ATP formed (%)*
Non-treated fragments	100
EDTA-treated fragments	0
EDTA-treated fragments + factor	27
EDTA-treated fragments + boiled factor (3 min, 100°)	0
EDTA-treated fragments + dialysed factor (24 h, 0°)	13
EDTA-treated fragments + stored factor (24 h, 0°)	15

* All contained 0.008 M $MgCl_2$.

TABLE III

SPECIFICITY OF THE COUPLING FACTOR

Reaction conditions as described under Table I, but with 10 μg of chlorophyll per ml. Control activities are indicated in brackets.

*Present during preincubation**	*Phosphorylation dependent upon*			
	Phenazine methosulfate (%)	*Ferricyanide (%)*	*FMN (%)*	*Menadione (%)*
Non-treated fragments	100 (536)	100 (59)	100 (54)	100 (52)
Non-treated fragments + factor	97	103	90	93
EDTA-treated fragments	1	1	4	3
EDTA-treated fragments + factor	15	6	4	1

* All contained 0.002 M $MgCl_2$.

The effect of EDTA in releasing a factor which is necessary for coupled phosphorylation has also been found in mitochondrial preparations[1-4]. JAGENDORF AND SMITH[9] reported earlier that treatment of chloroplasts with EDTA caused rapid uncoupling of the preparation. Preincubation in the presence of magnesium ions, the phosphorylating particles, and the coupling factor has also been reported to be essential for recoupling mitochondria[1]. It seems, therefore, that the coupling factor reported herewith may be similar in properties and function to at least one of the coupling factors reported for oxidative phosphorylation. Further details will be published elsewhere[10].

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