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Transfer of radioactivity from the prelabelled cell debris to the microsomal protein

The study of HENDLER[1], showing that mince of hen oviduct incorporates [^{14}C]amino acids into protein but homogenates of the same tissue do not incorporate the amino acids, suggested to us the possibility of the involvement of a factor similar to the prelabelled cell debris concerned in the incorporation of [^{14}C]amino acids into protein in silkworms' silk glands.

In fact, as shown in our previous paper[2], the ability to incorporate [1-^{14}C]glycine into the protein fraction of silk glands was decreased to about one hundredth by the homogenation of the glands.

Studies carried out using subcellular fractionation and incubation of silk glands essentially according to the methods of SHIMURA[3] for the incorporation of ^{14}C-labelled precursors into microsomal protein, with cell debris as the prelabelled source of ^{14}C, showed a marked increase in the radioactivity incorporated into protein. When [1-^{14}C]glycine was used as the source of radioactivity, the relative specific activity of the microsomal protein was less than 0.0001 %, whereas in the case of cell debris as precursors the value was generally around 20 %.

The complete reaction mixture contained approximately 5.8 mg E_1 protein, 7.8 mg E_2 protein and 3.2 mg E_3 protein; 16 μmoles ATP; 20 μmoles phosphocreatin; 8 μmoles $MgCl_2$; 120 μmoles KCl; 100 μmoles Tris–HCl (pH 8.0); 800 μmoles sucrose; 1.0 mg creatine kinase; 0.5 μmole GTP; and 19 amino acids (8 μmoles DL-alanine, 4 μmoles L-serine, 0.8 μmole L-tyrosine, 2 μmoles of DL-valine and DL-threonine, 1 μmole of L-aspartic acid, L-asparagine, L-phenylalanine and L-glutamic acid, 0.5 μmole of L-isoleucine, L-histidine, L-leucine, L-proline and L-arginine, 0.25 μmole of L-glutamine, L-cystine, L-methionine, L-tryptophan and L-lysine).

[2-^{14}C]Glycine-labelled silk glands cell debris was obtained by the following method: 15 min after the injection of 2 μC [2-^{14}C]glycine *per capita*, the silk glands

Abbreviations: ATP, adenosine triphosphate; GTP, guanosine triphosphate; Tris, Tris(hydroxymethyl)aminomethane.

TABLE I

TRANSFER OF RADIOACTIVITY FROM [^{14}C]CELL DEBRIS TO MICROSOMAL PROTEIN

System	*Incubation time (min)*	*Radioactivity transferred to microsomal protein counts/min/mg*
Complete system	0	23
Complete system	15	130
Complete system	30	114
Complete system	60	119
Complete system–E_3	30	146 (120*)
Complete system–Amino acids	30	53
Complete system–GTP	30	96
Complete system–ATP and ATP-regenerating system	30	55 (29**)
Complete system–E_1–E_2–E_3	30	

Cell debris: The precipitate of the centrifugation of silk-glands homogenate at 700 × *g* for 10 min. E_1 (microsomes): After removal of large particles by centrifugation at 14,000 × *g* for 30 min, the supernatant was acidified at pH 6.1 and recentrifuged at 8500 × *g* for 8 min. Sediment used. E_2 (incorporation enzymes): The supernatant of E_1 was adjusted at pH 4.9 and centrifuged at 8500 × *g* for 8min. Sediment used. E_3 (amino-acid-activating enzymes): The supernatant of E_2.

* Corrected for difference of protein concentration.

** Radioactivity contained in the supernatant after centrifugating at 1400 × *g* for 10 min.

were removed, and the cell-debris fraction was separated by the method of SHIMURA[3]. After thorough washings with 10% non-labelled glycine solution, the cell debris was twice homogenized in a Waring blender and centrifuged at 700 × *g* for 10 min. Then the pellet was rehomogenized with a Potter-Elvehjem-type homogenizer. Freezing and thawing was carried out in order to destroy any contaminating intact cells. Microscopic examination showed that the major component was nuclear debris without any intact cells. The specific activity was 547 counts/min/mg and a total of 43,000 counts/min was added to each vessel. The final volume of reaction mixtures was 2 ml. After adjusting the pH to 7.4–7.6, the mixtures were incubated at 37° under air and before the reaction was stopped by the addition of 4 ml 8% $HClO_4$, the incubated media were mixed with 2 ml of non-labelled 10% glycine solution to eliminate contamination from the surface of the denatured protein[3], and the ^{14}C-labelled cell debris was centrifuged off at 6000 × *g* for 10 min. Contamination remaining in the supernatant fraction was less than 0.5%. The microsomal fraction was obtained by centrifugation at 8500 × *g* for 8 min after acidification of reaction medium at pH 5.5 Further fractionation was carried out according to the method of HUGGINS AND COHN[4] to remove the phosphatidopeptide from the protein fraction. The radioactivity was measured in a windowless gas-flow counter. All results were expressed in counts/min/mg (ref. weight, 10 mg) according to the study of HENDLER[5].

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Electron-transport and phosphorylation inhibitor in *Tetrahymena* and evidence for its formation by a phospholipase

Preparations of *T. pyriformis* were reported to form by enzymic action a heat-stable agent (or agents) which inhibits various respiratory enzymes of rat-liver mitochondria and *Tetrahymena* homogenate[1]. It has now been shown that this agent inhibits oxidative phosphorylation, activates latent ATPase, and promotes swelling of rat-liver mitochondria. In addition, evidence implicating a phospholipase in its production has been obtained.

Particle preparations (made from homogenates of the GL strain), aged 24 h at 0° and then boiled to destroy the enzyme which produces the inhibitor, uncouple phosphorylation and depress O_2 uptake in the presence of succinate (Table I). Usually, preparations containing 30–50 μg N inhibit phosphorylation 100% and respiration 55–80%. Particle preparations, boiled when fresh to reduce inhibitor formation, also suppress phosphorylation and decrease O_2 uptake but much larger amounts are required (Table I). Crystalline bovine serum albumin prevents the effect of the GL

TABLE I

INHIBITION OF OXIDATIVE PHOSPHORYLATION AND OXYGEN UPTAKE OF LIVER MITOCHONDRIA BY A *T. pyriformis* PREPARATION AND PROTECTION BY ALBUMIN

The reaction mixture contained: 40 μmoles potassium phosphate buffer, pH 7.4; 20 μmoles $MgCl_2$; 5 μmoles ATP; 60 μmoles glucose; 140 K.M. units hexokinase; 100 μmoles sodium succinate; 0.042 μmole cytochrome *c*; and 0.28 mg rat-liver mitochondrial N in 63 μmoles sucrose; final volume, 2.8 ml; 0.2 ml 20% KOH in centre well; temp. 30°; duration of experiment 15 min plus 7 min equilibration; phosphate uptake assayed by change in inorganic P by method of LOWRY AND LOPEZ[2]. 7 day-old culture of *T. pyriformis* GL homogenized as described[1]; a portion was immediately boiled 20 min; both preparations were stored 24 h at 0° and the unboiled portion was then boiled 20 min. Both preparations were centrifuged 30 min at 105,000 × *g* to obtain the particles (1.1 mg N/ml).

Additions	*ΔO μatoms*	*ΔP μmoles*	*P/O*
None	6.0	10.8	1.8
0.025 ml GL particles, aged (0°) and boiled	4.9	6.5	1.3
0.05 ml GL particles, aged (0°) and boiled	3.1	0.5	0.2
0.1 ml GL particles, aged (0°) and boiled	2.1	0.5	0.2
0.2 ml GL particles, aged (0°) and boiled	0.6	0	0
0.05 ml GL particles, boiled and aged (0°)	6.8	11.0	1.6
0.1 ml GL particles, boiled and aged (0°)	6.1	8.6	1.4
0.2 ml GL particles, boiled and aged (0°)	4.1	3.3	0.8
0.3 ml GL particles, boiled and aged (0°)	3.9	0	0
0.05 ml GL particles, aged (0°) and boiled + 5 mg crystalline bovine albumin	6.8	10.9	1.6

Abbreviations: ATP, adenosine triphosphate; Tris, tris(hydroxymethyl)aminomethane.