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THE POSSIBLE ROLE OF ATP-DEPENDENT PROTEOLYSIS ON THE SOLUBILIZATION OF METHEMOGLOBIN REDUCTASE DURING RETICULOCYTE MATURATION

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SUMMARY: The ATP-dependent proteolytic system present in reticulocytes can release the active hydrophilic domain of cytochrome b_5 and NADH-cytochrome b_5 reductase from the endoplasmic reticulum, that in mature erythrocytes act as methemoglobin reductase.

In 1959 we showed that cytochrome b_5 and NADH-cytochrome b_5 reductase act as methemoglobin reductase (1). Passon and Hulquist (2) demonstrated the presence of those two electron carriers in the cytosol of reticulocytes and showed that they are responsible for the methemoglobin reductase activity. Both cytochrome b_5 and NADH-cytochrome b_5 are attached to the endoplasmic reticulum by a hydrophobic domain, with a hydrophilic catalytic domain which protrudes from the membrane (3,4), and the soluble cytochrome b_5 in mature erythrocytes is the free hydrophilic domain (5).

An ATP-dependent proteolytic system was found to be present in reticulocytes (6) and shown to be composed of a heat sensitive component and a 9 kDa heat stable polypeptide (7). This heat stable polypeptide, that was identified as ubiquitin, forms a covalent compound with the protein that acts as substrate, a reaction which requires ATP (8). This system has been implicated in the proteolysis of reticulocyte mitochondria after the action of lipoxygenase (9), the degradation of chemically modified protein (10) and in reticulocyte maturation (6).

The activity of this ATP-dependent proteolytic system has been measured by the release of free amino acids or acid soluble peptides (6,10,11). In this report we have shown that this system can act on liver microsomes releasing active cytochrome b_5 and NADH-cytochrome b_5 reductase, which catalyse the reduction of cytochrome c by NADH.

METHODS

Reticulocytes were isolated from rabbit blood after phenylhydrazine treatment, and used for the isolation of fraction I (heat stable) and fraction II (heat labile), by DEAE-cellulose fractionation and heat treatment (11). Both fractions were treated with 1 mg/mg of protein of activated charcoal (Darco-60) to remove nucleotides. Rat liver microsomes were

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Table I. Effect of ATP on the solubilization of cytochrome b_5 and NADH-cytochrome b_5 reductase by reticulocyte preparations

	ΔA_{550} /min/ml	
additions	no ATP+PEP	ATP+PEP
none	0.16	0.18
fraction I	0.18	0.22
fraction II	0.32	0.45
fraction I + fraction II	0.30	1.51

The incubation system contains in 1 ml: 9.8 mg of rat liver microsomal protein, 0.9 mg of fraction I protein, 1.0 mg of fraction II protein, 0.1 M tris buffer pH 7.5, 5 mM magnesium chloride, 10 U crystalline rabbit muscle pyruvate kinase, 0.5 mM ATP and 10 mM sodium phosphoenolpyruvate.

isolated by differential centrifugation (12). The suspension of microsomes, reticulocyte fractions and other components was incubated at 25°C for 1 hour and microsomes were precipitated with 8 mM calcium chloride (13) and removed by centrifugation at 70,000 g for 1 hour. The supernatant was treated with 10 mM EDTA and assayed for NADH-cytochrome c reductase (14).

RESULTS AND DISCUSSION

The release of cytochrome b_5 and NADH-cytochrome b_5 reductase from rat liver microsomes by rabbit reticulocyte fractions I and II is stimulated by ATP (Table I). The functional integrity of the cytochrome b_5 and its reductase is shown by their ability to catalyze the reduction of cytochrome c by NADH.

These results are the first indication that the ATP-dependent proteolytic system can release a functional enzyme, and suggest that this system may be involved in the release of the electron carriers which constitute the active methemoglobin reductase in mature erythrocytes.

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