AMINO ACID INCORPORATION BY MITOCHONDRIA OF THE ADRENAL CORTEX: THE EFFECT OF CHLORAMPHENICOL*

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Electron microscopic studies revealed that mitochondria of the adrenal cortex are very different in structure from mitochondria of other organs (Sabatini et al (1961); Lever, (1956). These mitochondria contain cholesterol, a major precursor in steroid hormone production, as well as the enzyme system which is described as rate-limiting in steroidogenesis and considered to be the site of action of adrenocorticotropic hormone (ACTH) (Halkerston et al 1961; Stone and Hechter, 1954; Karaboyas and Koritz, 1965; Davis and Garren, 1966).

Isolated mitochondria obtained from liver and heart have been shown to incorporate amino acids into protein (Rendi, 1959; Kroon, 1965; Wheeldon, Lehninger, 1966). Although chloramphenicol failed to inhibit microsomal protein synthesis in several different tissues from higher organisms (Nathans et al, 1962; Kroon, 1965), this antibiotic strongly inhibited the incorporation of amino acids into protein in mitochondria obtained from heart and liver (Rendi, 1959; Kroon 1965)

Because recent studies showed that inhibitors of protein synthesis blocked the stimulation of corticosterone production by ACTH (Ferguson, 1963; Garren, Ney, Davis, 1965) and Farese, (1964) demonstrated that chloramphenicol also inhibited this action of ACTH, the present study was undertaken.

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In this investigation a mitochondrial fraction isolated from the adrenal cortex was demonstrated to be active in the incorporation of amino acids into protein. The effect of chloramphenicol on this amino-acid incorporating system is compared with that obtained from microsomes of the adrenal cortex.

METHODS

Beef adrenals were obtained from the local abbatoir, and transported to the laboratory on ice. After trimming the fat from the capsules of the glands, the adrenals were sliced into sections and the medullae carefully removed. The adrenal cortices were minced and homogenized in 9 volumes of a medium containing: 0.25M sucrose, 0.05M Tris chloride (pH 7.6), 0.005M $MgCl_2$, and 0.025M KCl (Littlefield and Keller, 1957). After centrifugation at 600 xg for 10 minutes, the supernatant fluid was removed and centrifuged at 5000 xg for 10 minutes. The supernatant was discarded and the precipitated pellet was resuspended in 0.154M KCl and centrifuged at 5,000 xg for 10 minutes. The pellet was resuspended and washed two additional times in 0.154M KCl as above. The washed pellet was finally resuspended in 0.154M KCl to constitute the mitochondrial fraction used in the present studies. All experiments were carried out on freshly prepared mitochondria.

The microsomes were obtained from the same beef adrenal cortices which were used for the mitochondrial fraction by a method similar to that described by Maxwell, (1962) for liver microsomes. Amino-acid incorporation in this fraction was measured according to the Maxwell (1962) modification of the technique of Keller and Zamecnik, (1956). Additions of 1.0 mg microsomal protein and 3.0 mg protein of the pH 5 fraction were made to the incubation mixture which contained 0.35 M sucrose, 0.07M KCl, 0.05 M Tris buffer

pH 7.8, 1×10^{-3}M ATP, 6×10^{-3} M mercaptoethanol, 1×10^{-4} M GTP, 5×10^{-3}M sodium phosphoenolpyruvate, 20μg crystalline phosphoenol pyruvate kinase (Sigma Chemical Co.) 8×10^{-5} M, ^{14}C leucine, 1×10^{-4} M each of 19 ^{12}C amino acids minus the ^{14}C amino acid, 5×10^{-3} M $MgCl_2$. The final volume was 0.5 ml and the incubation was at 30°. The pH 5 fraction that was used in all studies was obtained from rat liver as described by Hoagland et al (1958).

The complete amino-acid incorporating system for the mitochondrial subfraction was the same as described above for the microsomes with the exception that the pH 5 fraction and the 19 C^{12} amino acids were routinely omitted unless otherwise specified in the experiment. Mitochondrial protein (1.5 mg) was added to a final volume of 0.5 ml and the incubation was at 37°. Protein was determined by the Lowry modification of the Folin-Ciocalteau method (Lowry et al, 1951). L-leucine-^{14}C uniformly labeled (specific activity 300 mc (mMole) was obtained from the New England Nuclear Corporation.

RESULTS

In the mitochondrial fraction, Fig. 1, the incorporation of leucine ^{14}C into protein insoluble in hot trichloroacetic acid was linear for approximately one hour and then declined in rate. These kinetics are similar to the findings reported by Wheeldon and Lehninger, (1966), but differs from that of Kroon, (1955) in which the amino acid incorporation was linear for at least 3 hours. The mitochondria used in their experiments were obtained from rat liver.

Mitochondria incorporated amino acids maximally in the presence of ATP and an ATP generating system (Table I).

TABLE I

Mitochondrial Amino Acid Incorporation

Experimental	cpm/mg protein
Complete system	381
Complete system + chloramphenicol	13
Complete system + RNA ase	286
No ATP, phosphoenolpyruvic acid or phosphoenolpyruvic kinase	200
Heated 55° for 10 minutes	10
+ pH 5 fraction	370

However, this effect of ATP on the activity was not constant. As has been described in other mitochondrial systems, incorporation of amino acids into protein by this adrenal fraction was not dependent upon the addition of transfer RNA and the enzymes contained in the "pH 5" fraction (e.g. Kroon, 1965). These factors are essential for microsomal amino acid incorporation. Again, unlike the microsomal system, treatment with ribonuclease either slightly decreased adrenal mitochondrial activity or had no effect on the system. Heating the mitochondrial preparation at 55° for 10 minutes before incubation, which was done at 37°, inactivated it completely. The addition of chloramphenicol (50μg/flask) almost completely inhibited the incorporation of ^{14}C leucine into the mitochondria (Table I, Fig. 1).

A more detailed description of the requirements of this mitochondrial amino-acid incorporating system from beef adrenals will be presented elsewhere. (Insert Fig. 1 and Fig. 2).

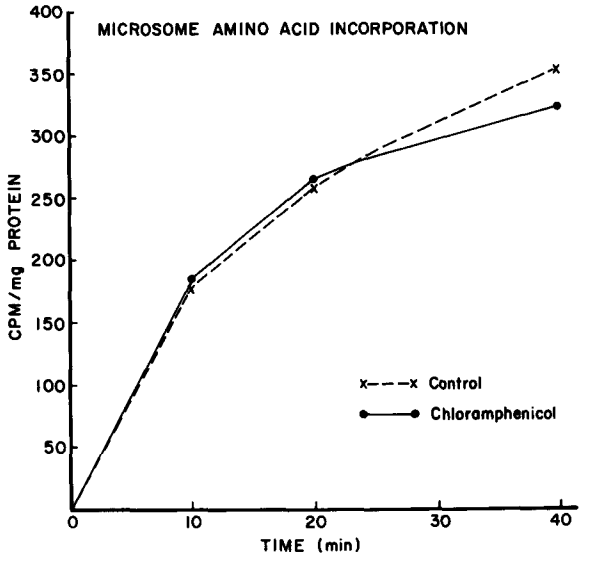

Fig. 1

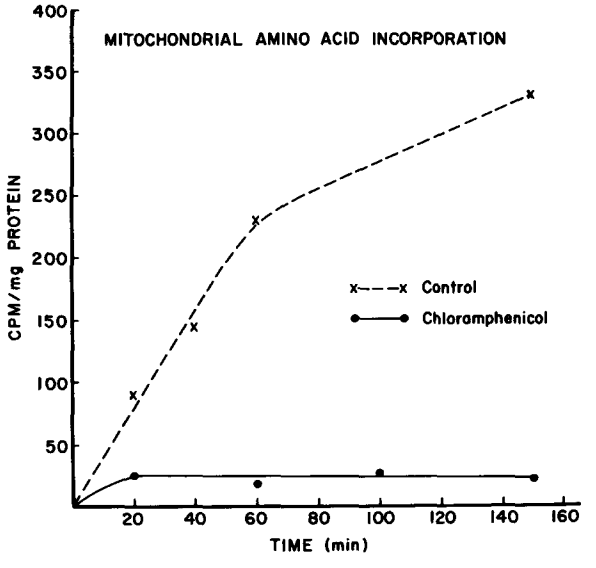

Fig. 2

In the microsomal system which was isolated from the same adrenal cortices as the mitochondrial fraction described above, chloramphenicol (50μg/flask) failed to inhibit ^{14}C-leucine incorporation (Fig. 2).

As argued by Wheeldon and Lehninger (1966), concerning their mitochondrial system from rat liver, the kinetics of the incorporation of amino acids by the adrenal mitochondrial fraction strongly suggests that the amino acid incorporation by mitochondria cannot be accounted for by contaminating bacteria (Fig. 1). If bacteria were solely responsible for the observed activity, a logarithmically increasing rate of incorporation would be expected. Additional evidence demonstrating that the activity observed was not bacterial in origin will be presented elsewhere.

DISCUSSION

These findings demonstrate that the adrenal cortex contains two distinct subcellular systems which are active in the incorporation of amino acids. The sensitivity of the mitochondrial subfraction of the adrenal cortex to chloramphenicol differentiates it from the microsomal system which is insensitive to this antibiotic. The failure of chloramphenicol to inhibit microsomal activity while completely blocking the activity of the mitochondrial subfraction, indicates that the mitochondrial fraction was not significantly contaminated by microsomes. Although the characteristics of the incorporation of amino acids by this fraction from the adrenal cortex are in line with those reported as isolated mitochondria from other tissue, additional purification procedures and verification by electron microscopy (which are in progress) must be performed before it is entirely certain that the activity of this fraction is completely mitochondrial in origin.

Present evidence suggests that the rate limiting reaction of corticosterone biosynthesis, (i.e. the transformation of cholesterol to 20 α hydroxycholesterol) is the site of action of ACTH (Stone and Hecter, 1954; Karaboyas and Koritz, 1965; Davis and Garren, 1966). This reaction occurs within the mitochondria, requires molecular oxygen, a carbon monoxide binding pigment (P-450) responsible for

oxygen activation and TPNH (Estabrook, Cooper, and Rosenthal, 1963; Harding, Wilson, Wong, and Nelson, 1965; Simpson and Boyd, 1966; Halkerston et al, 1961 Solomon et al, 1956; Constantopoulos et al, 1962; Shimizu et al, 1962). It has also been suggested that the TPNH utilized in this reaction is generated from intramitochondrial sources (Koritz, 1966).

The present study and the report that chloramphenicol inhibits the stimulation of corticosterone synthesis by ACTH (Farese, 1964), stimulates the speculation that ACTH action is mediated at least in part through the synthesis of a protein or proteins by the mitochondria of the adrenal cortex. This postulated protein could either regulate or be directly involved in any aspect of the intramitochondrial enzyme complex that transforms cholesterol to 20 α hydroxycholesterol, i.e., the desmolase that cleaves the cholesterol side chain, the generation of TPNH, or the P-450 activation of molecular oxygen.

Caution is required in relating mitochondrial protein synthesis to ACTH action when this is based solely on the findings that chloramphenicol inhibits both steroidogenesis and mitochondrial protein synthesis. Nevertheless, these observations suggest an interesting speculation concerning ACTH action. The hypothetical regulatory protein or its mechanism of action has not been elucidated.

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