Vol. 130, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1985 Pages 407-411

STUDIES ON RHODANESE SYNTHESIS IN BOVINE ADRENOCORTICAL CELLS

Vijayakumar Boggaram*, Paul Horowitz** and Michael R. Waterman*

*Department of Biochemistry, University of Texas
Health Science Center, Dallas, TX 75235
and
**Department of Biochemistry, University of Texas
Health Science Center, San Antonio, TX 78284

Received May 20, 1985

The synthesis of adrenodoxin, a mitochondrial iron-sulfur protein required for adrenocortical steroidogenesis, is known to be regulated chronically by ACTH. Rhodanese, also a mitochondrial enzyme, is thought to be required for synthesis of iron-sulfur centers, such as those contained in adrenodoxin. In this study it has been found that rhodanese synthesis and activity are not regulated by ACTH, under the same conditions whereby ACTH induces adrenodoxin synthesis. In addition, unlike adrenodoxin, rhodanese is found to be synthesized in the mature form rather than as a higher molecular weight precursor protein.

The mechanisms involved in the in vivo assembly of iron-sulfur centers of iron-sulfur proteins are largely undiscovered. Sulfur transferases have been suggested to be involved in the assembly of these centers (1,2), and recent studies have demonstrated in reconstitution experiments that the mitochondrial enzyme rhodanese (3) mediates the transfer of sulfur from radiolabeled thiosulfate to various apo iron-sulfur proteins leading to enzymatically active reconstituted proteins (4-7).

Adrenocortical mitochondria contain an iron-sulfur protein, adrenodoxin, which is an essential electron transfer component of mitochondrial steroid hydroxylase complexes (8,9). ACTH regulates steroidogenesis in the adrenal cortex as a result of both acute and chronic actions (10). The chronic action of ACTH on the adrenal cortex involves the regulation of the levels of steroid hydroxylase cytochromes P-450 and their associated electron transfer components (11). This action of ACTH results from a cyclic AMP-mediated increase in the rate of synthesis of cytochromes P-450 and adrenodoxin as determined in bovine adrenocortical cells in primary culture (12). The increase

0006-291X/85 $1.50

in apo-adrenodoxin synthesis caused by ACTH suggests that an increase in the activity of the enzyme system(s) responsible for the assembly of iron-sulfur centers of adrenodoxin might also occur. In view of the recent studies which indicate that rhodanese mediates the transfer of sulfur to apo iron-sulfur proteins, it was of interest to examine the effect of ACTH on the synthesis and activity of rhodanese. In the present study we have investigated the effect of ACTH on the synthesis and activity of this enzyme in bovine adrenocortical cells in primary culture and compared these results to the effect of ACTH on the synthesis of adrenodoxin.

MATERIALS AND METHODS

Procedures for cell culture, radiolabeling of cellular proteins and immunoisolation of radiolabeled enzymes, isolation of RNA, in vitro translation of RNA and immunoisolation of newly synthesized enzymes from total translation products were carried out as previously described (12). For the preparation of mitochondria, cells were homogenized in 25 mM Hepes, pH 7.4, containing 0.25 M sucrose. Mitochondria were obtained by centrifugation of 800 x g supernatant for 20 min at 10,000 x g. Cortisol in the culture medium was measured by direct radioimmunoassay using an antibody prepared against cortisol-3-carboxymethyloxime-bovine serum albumin. Sulfur transferase activity of rhodanese was determined by following the rate of formation of thiocyanate from thiosulfate and cyanide according to Sorbo (13). The antibody specific for adrenodoxin (14) was prepared against purified bovine adrenodoxin kindly supplied to the laboratory by Dr. J.D. Lambeth. Purified bovine rhodanese was prepared as previously described (15). Antibody against rhodanese was prepared in rabbits by classical procedures using the purified protein in Freund's complete adjuvant.

RESULTS AND DISCUSSION

Treatment of bovine adrenocortical cells in monolayer culture with ACTH for up to 60 hours did not increase the incorporation of [^{35}S]-methionine into immunoprecipitable rhodanese while there was a significant increase in the incorporation of [^{35}S]-methionine into immunoprecipitable adrenodoxin. Quantitation of autoradiographs prepared from adrenodoxin and rhodanese immunoisolates is shown in Fig. 1. In the case of adrenodoxin the rate of synthesis reached a maximum 36 hours following the initiation of treatment with ACTH, as observed previously (14).

Mitochondria isolated from cells maintained in the presence or absence of ACTH were assayed for rhodanese activity. Mitochondria obtained from ACTH treated cells did not show any increase in the activity of rhodanese compared to

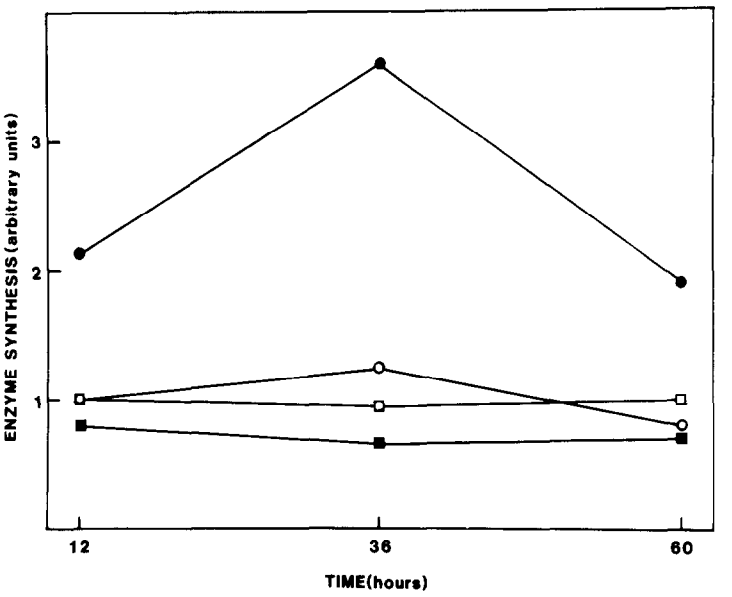


Fig. 1 - Effect of ACTH on the rate of synthesis of rhodanese [control (□), ACTH (■)] and adrenodoxin [control (○), ACTH (●)]. Cells were maintained for various periods of time up to 60 h in the absence or presence of ACTH (1 μM). At times indicated, they were radiolabeled with [^{35}S]methionine and newly synthesized rhodanese and adrenodoxin were immunoisolated from 2.0 x 10^6 cpm.

those obtained from cells not treated with ACTH (Table I). The activity of rhodanese in bovine adrenocortical cells in primary culture is lower than that found in mitochondria from freshly obtained bovine adrenal gland, but ACTH treatment had no effect. Determination of cortisol in media of cells treated with or without ACTH indicated that ACTH caused more than 10-fold increase in the production of cortisol by bovine adrenocortical cells (Table I). Thus, the steroidogenic pathway of these cells was responsive to ACTH as was the

Table I

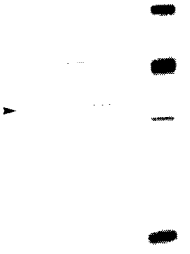
Effect of ACTH on Rhodanese Activity and Cortisol Production in Bovine Adrenocortical Cells

	Rhodanese Activity (Units x mg^{-1} Protein)			Cortisol (nmoles x 36 h^{-1} per dish)
Treatment	12 h	36 h	60 h	36 h
Control	0.70	0.608	0.433	10.34
ACTH	0.55	0.336	0.35	121.00

Gland activity = 2.45 units x mg^{-1}

synthesis of adrenodoxin, even though rhodanese synthesis and activity were not. Other effectors capable of regulating cytochrome P-450 and adrenodoxin synthesis such as dibutyryl cAMP (12) and cholera toxin (16) were also ineffective at regulating rhodanese synthesis.

As many mitochondrial proteins (17) including adrenodoxin (18) are synthesized as higher molecular weight precursors it was also of interest to investigate the possibility that rhodanese might also be synthesized as a higher molecular weight precursor. Immunoisolation of newly synthesized rhodanese from total translation products directed by RNA isolated from bovine adrenocortical cells in culture followed by electrophoretic analysis (SDS-PAGE) in different gel systems (10%, 12.5%, 15% and 7.5%-12.5% exponential gradient) showed no indication of the synthesis of a higher molecular weight form of rhodanese compared to newly synthesized rhodanese immunoisolated from labeled cellular protein or the purified protein (Fig. 2). Identity of this translation product as rhodanese was confirmed by competition of the immunoisolation by



1 2 3 4 5

Fig. 2 - Immunoisolation of rhodanese from radiolabeled adrenocortical cell lysates and total RNA translation products (2.0×10^6 cpm in all cases). Lane 1, newly synthesized rhodanese from cell lysate; Lane 2, identical sample to lane 1 except immunoisolation was competed by addition of 30 µg purified, unlabeled rhodanese; Lane 3, newly synthesized rhodanese from RNA translation products; Lane 4, identical sample to lane 3 except immunoisolation was competed by 30 µg purified, unlabeled rhodanese; Lane 5, molecular weight markers (in descending order, phosphorylase B (92,500), albumin (69,000), ovalbumin (46,000), carbonic anhydrase (30,000) and cytochrome c (12,300). The arrow indicates the migration of purified beef liver rhondanese. A molecular weight of 34,000 was calculated for newly synthesized and purified rhodanese from such gels.

purified rhodanese. Thus, rhodanese, which is localized in the mitochondrial matrix (19), is not synthesized as a higher molecular weight precursor, unlike many, but not all, matrix enzymes (17).

Since rhodanese has been implicated in the generation of the labile sulfur of iron-sulfur proteins it was expected that ACTH might increase the rate of synthesis and consequently the activity of rhodanese, as it does in the case of adrenodoxin. The present results indicate that the existing rhodanese activity of bovine adrenocortical cells is sufficient to generate functional adrenodoxin from apo-adrenodoxin. This is confirmed by the observation of increased amounts of adrenodoxin iron-sulfur centers in adrenocortical cells in response to ACTH treatment as determined by electron spin resonance spectroscopy (14).

ACKNOWLEDGMENT

This work was supported in part by Grant I-624 from The Robert A. Welch Foundation and USPHS grant AM 28350.

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