STUDIES ON THE IMMUNOCHEMICAL AND FUNCTIONAL SIMILARITIES AMONG IRON-SULFUR PROTEINS INVOLVED IN MITOCHONDRIAL STEROID HYDROXYLATIONS

Jeffrey Baron

The Toxicology Center, The Department of Pharmacology,
The University of Iowa, Iowa City, Iowa 52242

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SUMMARY: The effects of a goat anti-adrenodoxin immunoglobulin fraction on the NADPH- and NADH-dependent electron transport sequences in the mitochondria of steroidogenic tissues have been examined. The NADPH-cytochrome c reductase activities of sonicated mitochondrial preparations derived from bovine adrenal cortex and rat adrenals and ovaries were inhibited in a similar manner in the presence of this antibody, while the inhibition of this activity in rat testicular mitochondrial preparations was less pronounced. The NADH-dependent reduction of cytochrome c catalyzed by these mitochondrial preparations was not affected by the antibody. These results indicate that, while they may not be identical, the iron-sulfur proteins involved in mammalian mitochondrial steroid hydroxylations exhibit immunochemical and functional similarities.

The oxidative cleavage of the cholesterol side-chain to form pregnenolone is the initial step in the biosynthesis of steroid hormones in the adrenal, testis, ovary and placenta. The enzyme systems catalyzing this reaction are associated with the mitochondrial fraction in these tissues and require NADPH and molecular oxygen for activity (1). The cholesterol side-chain cleavage systems of bovine adrenocortical (2), rat ovarian (3) and human placental (4) mitochondria have each been resolved into three protein components: cytochrome P-450, the terminal oxidase; an NADPH-flavoprotein dehydrogenase; and an iron-sulfur protein which, in the case of the adrenals, has been named adrenodoxin. These systems thus appear to be similar to the 11β-hydroxylase system of bovine adrenocortical mitochondria which has also been resolved into three components by Omura et al. (5). Although the cholesterol side-chain cleavage system of testicular mitochondria has not been resolved into its components, these mitochondria do contain an iron-sulfur protein which can replace adrenodoxin in the 11β-hydroxylase system of bovine adrenocortical mitochondria (6).

In the 11β-hydroxylase system of bovine adrenocortical mitochondria, Omura et al. (5) proposed that adrenodoxin functions as an electron transfer intermediate between the reduced flavoprotein dehydrogenase and oxidized cytochrome P-450. More recent studies employing an antibody to adrenodoxin demonstrated the requirement for adrenodoxin in the NADPH-dependent reductions of both cytochrome P-450 and cytochrome c as well as in the 11β-hydroxylation of deoxycorticosterone catalyzed by bovine adrenocortical mitochondria (7).

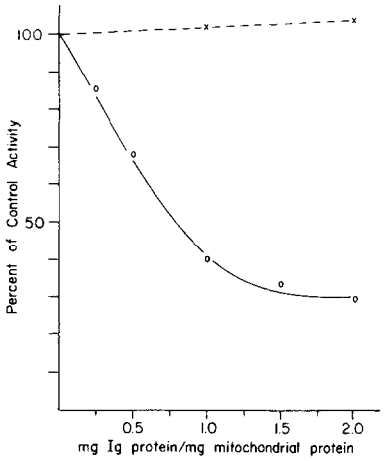

Figure 1: Titration of NADPH-cytochrome c reductase activity of bovine adrenocortical mitochondria by goat anti-adrenodoxin immunoglubulin.

Bovine adrenocortical mitochondria were sonicated for 4 minutes in 0.5-minute intervals. Each 2.2-ml reaction mixture contained 36.4 μM cytochrome c, 0.91 mM KCN, 0.1 mg of mitochondrial protein and 50 mM Tris-chloride buffer, pH 7.4. The reactions were initiated by the addition of 90 μM NADPH. Anti-adrenodoxin and preimmune immunoglobulin (Ig) were substituted for buffer in the indicated concentrations. The solid line represents the activities in the presence of anti-adrenodoxin immunoglobulin, and the dashed line represents activities in the presence of preimmune immunoglobulin. In the absence of immunoglobulin, NADPH-cytochrome c reductase activity was 60.7 nmoles cytochrome c reduced/min/mg protein.

It is generally assumed that the iron-sulfur proteins in other steroidogenic tissues function in a comparable manner in the side-chain cleavage of cholesterol catalyzed by the mitochondria of these tissues. This communication summarizes the initial results employing an antibody to adrenodoxin to examine the immunochemical and functional similarities among the iron-sulfur proteins of adrenal, ovarian and testicular mitochondria.

METHODS

Bovine adrenocortical mitochondria were prepared according to the method of Simpson and Boyd (8). Adrenals, ovaries and testes were removed from 140-160 g Holtzman rats, and the mitochondria were prepared by the same method. For studies employing testicular mitochondria, male rats received 500 units of human chorionic gonadotropin (Squibb) subcutaneously every 48 hours and were sacrificed 24 hours after the last injection. The anti-adrenodoxin and preimmune immunoglobulin fractions were prepared from immune and preimmune goat serum, respectively, by ammonium sulfate fractionation. Protein was determined by the biuret reaction (9). The activities of NADPH- and NADH-cytochrome c reductases were determined as previously described (7) after

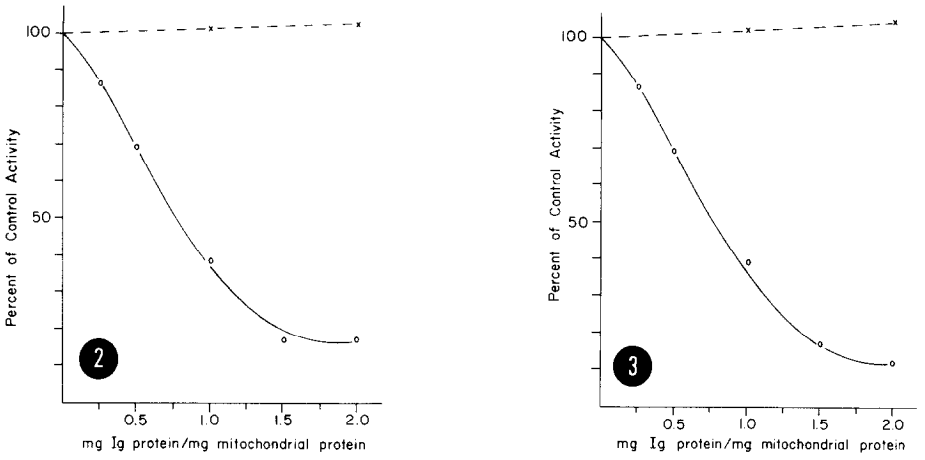

Figure 2: Titration of NADPH-cytochrome c reductase activity of rat adrenal mitochondria by goat anti-adrenodoxin immunoglobulin.

The conditions were the same as those described for Figure 1. In the absence of immunoglobulin, NADPH-cytochrome c reductase activity was 90.0 nmoles cytochrome c reduced/min/mg protein.

Figure 3: Titration of NADPH-cytochrome c reductase activity of rat ovarian mitochondria by anti-adrenodoxin immunoglobulin.

The conditions were the same as those described for Figure 1. In the absence of immunoglobulin, NADPH-cytochrome c reductase activity was 64.1 nmoles cytochrome c reduced/min/mg protein.

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the mitochondria had been sonicated for a total of 4 minutes using a Branson model W185 Sonifier at an output of 55 watts (20 kc).

RESULTS AND DISCUSSION

The immunization of a goat with homogeneous adrenodoxin isolated and purified from bovine adrenocortical mitochondria elicits the production of an antibody to the iron-sulfur protein. As can be seen in Figure 1, the addition of the goat anti-adrenodoxin immunoglobulin fraction to sonicated bovine adrenocortical mitochondria produced an inhibition of the NADPH-cytochrome c reductase activity of these mitochondria. No diminution of this activity was observed in the presence of the preimmune immunoglobulin fraction. Unlike its inhibitory effect on NADPH-cytochrome c reductase activity, the data in Table 1 show that the antibody was without effect on the activity of NADH-cytochrome c reductase in bovine adrenocortical mitochondria, an activity which does not require adrenodoxin (5). These results are in agreement with the previous studies of Baron et al. (7) in which an anti-adrenodoxin γ-globulin fraction derived from immune rabbit serum was employed. It was

Table 1. Lack of effect of goat anti-adrenodoxin immunoglobulin (Ig) on NADH-cytochrome c reductase activities of the mitochondria of steroidogenic tissues.[a]

Tissue	mg Ig protein/mg mitochondrial protein	nmoles cyt c reduced/min/ mg protein	Percent of control activity
Bovine adrenal cortex	0	346.8	100
	2.0 (immune)	369.4	107
	2.0 (preimmune)	348.7	101
Rat adrenal	0	59.0	100
	2.0 (immune)	58.8	100
	2.0 (preimmune)	56.6	96
Rat ovary	0	58.6	100
	2.0 (immune)	58.9	101
	2.0 (preimmune)	58.6	100
Rat testis	0	95.5	100
	2.5 (immune)	95.8	100
	2.5 (preimmune)	95.4	100

[a] The conditions were the same as those described for Figures 1-4, except that the reactions were initiated by the addition of 90 μM NADH.

found, however, that the goat immunoglobulin fraction was 5-10 times more inhibitory to NADPH-cytochrome c reductase activity than was an equal protein concentration of the rabbit γ-globulin.

The goat anti-adrenodoxin immunoglobulin fraction was also found to inhibit the NADPH-cytochrome c reductase activity catalyzed by rat adrenal mitochondria (Figure 2). Similar to the results obtained with bovine adrenocortical mitochondria, no inhibition of NADH-cytochrome c reductase activity of rat adrenal mitochondria was observed in the presence of the antibody as can be seen in Table 1. Although the extent of inhibition differs, the titration curves in Figures 1 and 2 are strikingly similar and demonstrate that the iron-sulfur proteins involved in mitochondrial NADPH-dependent electron transport in the adrenals of these two species exhibit immunochemical similarity. Moreover, these results further indicate the functional similarity of "adrenodoxin" in rat and bovine adrenals.

The data presented in Figure 3 show that, analogous to the results

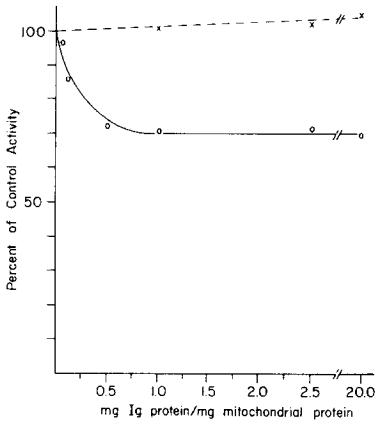

Figure 4: Titration of NADPH-cytochrome c reductase activity of HCG-treated rat testicular mitochondria by anti-adrenodoxin immunoglobulin.

The conditions were the same as those described for Figure 1, except that 0.5 mg of mitochondrial protein was present in each reaction mixture. In the absence of immunoglobulin, NADPH-cytochrome c reductase activity was 9.0 nmoles cytochrome c reduced/min/mg protein.

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obtained with rat and bovine adrenal mitochondria, the anti-adrenodoxin immunoglobulin fraction was capable of inhibiting the activity of NADPH-cytochrome c reductase of rat ovarian mitochondria. Indeed, this titration curve is almost identical to that obtained with rat adrenal mitochondria (Figure 2). As in adrenal mitochondria, the antibody did not alter NADH-cytochrome c reductase activity (Table 1). Thus, the iron-sulfur protein of rat ovarian mitochondria appears to be immunochemically and functionally similar to the comparable protein in adrenal mitochondria.

Mason et al. (10) have reported that, although testicular mitochondria of untreated rats contain little or no adrenodoxin-like iron-sulfur protein, treatment of rats with human chorionic gonadotropin (HCG) results in the increased synthesis of such an iron-sulfur protein. In agreement with these observations, the NADPH-cytochrome c reductase activity of mitochondria prepared from the testes of untreated rats was extremely low and was not consistently inhibited by the anti-adrenodoxin immunoglobulin fraction. However, when mitochondria derived from the testes of rats treated with HCG were studied, NADPH-cytochrome c reductase activity was elevated and inhibition of NADPH-cytochrome c reductase activity in the presence of the anti-adrenodoxin immunoglobulin fraction was observed (Figure 4). No inhibition of the NADH-dependent reduction of cytochrome c was found under the same conditions (Table 1). It is readily apparent that the titration curve obtained with rat testicular mitochondria (Figure 4) differs greatly

from those for adrenal and ovarian mitochondria (Figures 1-3). These observations may be due to one of the following possibilities: (a) the testicular iron-sulfur protein may exhibit only minimal immunochemical similarity with the adrenal and ovarian iron-sulfur proteins and might thus be more resistent to the inhibitory effects of the antibody; (b) a portion of the NADPH-cytochrome c reductase activity of rat testicular mitochondria may not involve the iron-sulfur protein; or (c) the iron-sulfur protein may be differently situated in the testicular mitochondrial membrane in comparison with the iron-sulfur proteins of the adrenal and ovary, and thus the relatively large antibody molecule may not be able to react with all of the testis iron-sulfur protein molecules which may be embedded in the mitochondrial membrane. This latter possibility is the most likely explanation for the inability of the antibody to inhibit completely the NADPH-cytochrome c reductase activity of sonicated bovine adrenocortical mitochondria while it can inhibit completely the activity of the reductase system solubilized from these mitochondria (7). Additional studies will undoubtedly define in greater detail the immunochemical and functional similarities among the iron-sulfur proteins involved in steroid hydroxylations occurring in the mitochondria of mammalian steroidogenic tissues.

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