DISCRIMINATION BETWEEN ASCORBATE:FERRICYTOCHROME b_5 OXIDOREDUCTASE AND THE CYANIDE-SENSITIVE FACTOR OF ACYL-CoA DESATURASE

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SUMMARY

By induction with high-carbohydrate diet it could be shown that ascorbate:ferricytochrome b_5 oxidoreductase (EC 1.10.2.1) and the cyanide-sensitive factor of the acyl-CoA desaturase system (EC 1.14.99.5) are not identical.

INTRODUCTION

Microsomal ascorbate:ferricytochrome b_5 oxidoreductase (EC 1.10.2.1) (1) has previously been described (2). It has been shown that the enzyme is neither identical with cytochrome b_5, nor with NADH:ferricytochrome b_5 oxidoreductase (EC 1.6.2.2), nor with NADH:monodehydro-L(+)-ascorbate oxidoreductase (EC 1.6.5.4) (1,3). Ascorbate:ferricytochrome b_5 oxidoreductase has cytochrome b_5 as one of its substrates, as has the cyanide-sensitive factor of the acyl-CoA desaturase (EC 1.14.99.5) (4-7). Furthermore L(+)-ascorbate functions as an electron donor in the acyl-CoA desaturase system (5,8,9). Thus the need arose to distinguish clearly between these two enzymes.

Oshino and Sato (8) have shown an increased synthesis of

the cyanide-sensitive factor in rat liver, after the animals were starved and refed on a high-carbohydrate diet. This finding suggested a simple method for testing the identity of the two enzymes in liver.

MATERIALS AND METHODS

Male rats (Wistar AF/Han) were fed on a standard diet (diet 1314 from Altromin, Lage Lippe) except for the induction experiments. Water was given ad libitum. Cytochrome b_5 without the hydrophobic domain was prepared from rat liver microsomes by tryptic digestion, based on the methods of Strittmatter and Velick (10) and Kajihara and Hagihara (11). The cyanide-sensitive factor was induced according to the method of Oshino and Sato (5). Liver microsomes were prepared as described by Weber et al. (12).

The activities of the ascorbate:ferricytochrome b_5 oxidoreductase and of the acyl-CoA desaturase were determined from the maximum velocity of the oxidation of ferrocytochrome b_5 according to Weber et al. (12) and Oshino and Sato (13), respectively. The assay for the acyl-CoA desaturase was carried out with p-cresol as substrate. The concentration of cytochrome b_5 in the assay mixture was adjusted to 1μM with isolated cytochrome b_5.

The two tailed t-test was used for the statistical comparison of the mean values of samples. The value of t and the number of degrees of freedom were calculated according to the approximations of Welch (14) since the samples had unequal variances. Differences were regarded as significant if $p < 0.01$.

RESULTS AND DISCUSSION

Table I shows the results of induction and control experiments. When evaluating the enzyme activities, one has to consider two possible sources of error that could be responsible for too high values:

1. The oxidation of ferrocytochrome b_5 in the presence of the enzyme and simultaneously in the absence of the second substrate, monodehydro-L(+)-ascorbate or p-cresol, respectively. This will be called apparent autoxidation. The apparent autoxidation is negligible when using sodiumdithionite as reducing agent. When using NADH as reducing agent, the apparent autoxi-

Table I : Specific Activity $\left[\frac{\text{nMol Cyt } b_5 \text{ ox.}}{\text{min x mg Protein}}\right]$ in the Presence of:

	Body Mass g	Induction by Carbohydrate	Number of Experiments	a (Apparent Autoxidation of Cyt b_5)	b p-Cresol (App. Autox. considered)	c p-Cresol (App. Autox. not consid.)	d Monodehydro-L(+)-ascorbate	Statistics (t-Test) *:$p < 0.01$
A	80 - 120	-	5	0.55±0.08+)	1.23±0.54	1.78±0.60	9.31±1.84	Ab/Bb: *
B	80 - 120	+	3	0.36±0.04	4.18±0.89	4.54±0.90	7.94±1.49	Ac/Bc: *
C	280 - 320	-	4	0.40±0.03	0.69±0.37	1.09±0.37	9.45±0.50	Cb/Db: *
D	280 - 320	+	4	0.42±0.04	4.07±1.49	4.49±1.50	9.57±1.17	Cc/Dc: *

+ mean value ± S.D.

0.1M phosphate, pH 7.4; 1.5mg microsomal protein/3ml; 1µM cytochrome b_5.

a,c : 32µM NADH.
d : 3.8mM sodiumdithionite.
c : 1mM p-cresol.
d : 12.5mM L(+)-ascorbate, and 12.5mM didehydro-L(+)-ascorbate.

dation is no longer negligible. It is uncertain, however, if there is a significant apparent autoxidation under the conditions of the enzyme assay. When using NADH as reducing agent, the enzyme activities were always evaluated both with and without consideration of the apparent autoxidation.

2. The oxidation of ferrocytochrome b_5 by a nonenzymatic reaction with the second substrate. p-Cresol does not cause such a nonenzymatic oxidation. Isolated reduced cytochrome b_5, however, is oxidized nonenzymatically by monodehydro-L(+)-ascorbate (15). As mentioned above isolated cytochrome b_5 was added to the assay mixture. The maximum velocity of the nonenzymatic oxidation of the added cytochrome b_5 was determined and taken into account when evaluating the enzyme activity. The nonenzymatic reoxidation of microsomal cytochrome b_5 is negligible. This can be deduced as follows. The enzyme activity is determined without addition of isolated cytochrome b_5, using different concentrations of monodehydro-L(+)-ascorbate. When plotting the results according to Lineweaver and Burk (16), one obtains a straight line only if the nonenzymatic oxidation is not considered.

As expected, the specific activity of the acyl-CoA desaturase in rat liver microsomes can be increased significantly by the diet described above. The specific activity of the ascorbate:ferricytochrome b_5 oxidoreductase, however, is not increased by the same treatment. Since the increased activity of the acyl-CoA desaturase is caused by an increased synthesis of the cyanide-sensitive factor (8), the activity of the ascorbate:ferricytochrome b_5 oxidoreductase must be caused by a different enzyme protein. The isolation of the cyanide-sensitive

factor (17) was unnecessary since the problem could be clearly resolved by the experiments presented.

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