OXIDATION OF ω-HYDROXYLATED FATTY ACIDS AND STEROIDS BY SS-ISOENZYME OF LIVER ALCOHOL DEHYDROGENASE

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Received February 25,1974

Summary. The SS-isoenzyme of alcohol dehydrogenase from horse liver was found to be active towards ω1- and ω2-hydroxylated fatty acids, an ω-hydroxylated steroid, ethanol and a 3β-hydroxysteroid. The main part of all these activities disappeared after carboxymethylation of a cysteine residue at the active site of $LADH_{SS}$. The ω-hydroxyfatty acid dehydrogenase activity of $LADH_{SS}$ was of similar magnitude as that of $LADH_{EE}$ whereas the ω-hydroxysteroid dehydrogenase activity of $LADH_{SS}$ was considerably higher than that of $LADH_{EE}$.

Alcohol dehydrogenase from horse liver contains three major isoenzymes, $LADH_{EE}$, $LADH_{ES}$ and $LADH_{SS}$. The most obvious catalytic difference between $LADH_{SS}$ and $LADH_{EE}$ is that $LADH_{SS}$ but not $LADH_{EE}$ is active towards 3β-hydroxysteroids (1, 2). Both $LADH_{SS}$ and $LADH_{EE}$ possess ethanol dehydrogenase activity. In spite of the drastic difference in catalytic activity between $LADH_{EE}$ and $LADH_{SS}$ the structure of the E- and S-subunits are highly similar and only six amino acids have been found to be different in the 374-residue protein chains (3). In previous work it was shown that alcohol dehydrogenase prepared from horse liver is active towards ω-hydroxylated fatty acids and steroids (4, 5). After carboxymethylation of a single cysteine residue at the active site of the enzyme the activities towards ω-hydroxylated fatty acids and steroids as well as ethanol were lost to the same extent, proving that alcohol dehydrogenase itself and not contaminating

enzymes was responsible for the activities (5). The alcohol dehydrogenase preparation used in that study (5) consisted almost exclusively of the isoenzyme $LADH_{EE}$. In the present work the possibility that the isoenzyme $LADH_{SS}$ is also active towards ω-hydroxylated fatty acids and steroids has been studied.

EXPERIMENTAL PROCEDURE

$LADH_{SS}$ and $LADH_{EE}$ were prepared from horse liver (6). Carboxymethylation of $LADH_{SS}$ with ^{14}C-iodoacetate was performed at $4^{o}C$ for 24 h with the protein in solution (1-2 mg/ml in 0.05 M Tris-Cl, pH 8.5, rather than in crystalline state, which was previously used for modification of $LADH_{EE}$ (5)). The specificity of incorporation of ^{14}C was controlled by autoradiography of tryptic peptide maps of the carboxymethylated protein. A 17-fold excess of reagent gave almost complete modification of cys-46, with much less substitution on other residues. The excess reagent was removed by dialysis against several changes of 0.05 M Tris-Cl, pH 8.5. The carboxymethylation was found to decrease the rate of oxidation of ethanol (7) by $LADH_{SS}$ by about 90%.

The ω1-hydroxyfatty acid dehydrogenase activity was assayed as described previously (5) using 18-hydroxystearic acid as substrate. No aldehyde dehydrogenase was added to the incubation mixture and a 0.1 M glycine buffer, pH 10.0, was used as incubation medium. The final volume of the incubation mixture was 3 ml. The ω2-hydroxyfatty acid dehydrogenase activity was assayed as described previously (5) using 17-L- or 17-D-hydroxystearic acid as substrate. The 17-L- and 17-D-hydroxystearic acids were generous gifts of Professor Tulloch (Prairie Regional Laboratory, Saskatchewan, Canada). ω-Hydroxysteroid dehydrogenase activity was assayed with tritium-labeled 5β-choles-

tane-3α,7α,12α, 26-tetrol as substrate (5). Glycine buffer, pH 10.0, was used as incubation medium and no aldehyde dehydrogenase was added (cf. ref. 5). 3β-Hydroxysteroid dehydrogenase activity was assayed under the same conditions as ω2-hydroxyfatty acid dehydrogenase activity except that 3β-hydroxy-5β-androstan-17-one, 50 µg dissolved in 25 µl acetone, was used as substrate. The steroid was obtained from Sigma Chemical Co. (St. Louis, Missouri). Incubations were terminated by addition of ethanol to the incubation mixture. The mixtures were then diluted with water, acidified and extracted with ether. The ether extracts were washed with water until neutral. After evaporation of the ether the residue was directly subjected to gas-chromatography using a 1% QF-1 column. Under the conditions employed there was a good separation between the incubated steroid (3β-hydroxy-5β-androstan-17-one) and the product (5β-androstane-3,17-dione). The product 5β-androstane-3,17-dione was also identified by combined gas chromatography - mass spectrometry using an LKB 9000 equipped with a 1.5% SE-30 column.

RESULTS

ω-Hydroxyfatty acid dehydrogenase activity.

$LADH_{SS}$ was found to catalyze conversion of 18-hydroxystearic acid into 1,18-octadecadioic acid in glycine buffer, pH 10.0. The rate of oxidation was about 0.3 µmoles/min/mg protein. The reaction was linear with enzyme up to about 3 µg of $LADH_{SS}$ and with time up to about 15 min. The enzyme was saturated with substrate and the apparent K_m was calculated to be 2-3 x 10^{-5}M.

ω2-Hydroxyfatty acid dehydrogenase activity.

$LADH_{SS}$ catalyzed oxidation of 17-L-hydroxystearic acid into

17-oxostearic acid at a rate of about 1 μmole/min/mg protein. With the 17-D-enantiomer of 17-hydroxystearic acid the activity decreased with about 90%. The oxidation of 17-L-hydroxystearic acid was linear with enzyme up to about 6 μg of $LADH_{SS}$ and with time for at least 20 min. The enzyme was saturated with substrate and the K_m was calculated to be about 2×10^{-5}M. The reaction was inhibited by 3 mM ethanol by about 50%.

ω-Hydroxysteroid dehydrogenase activity.

$LADH_{SS}$ was found to catalyze oxidation of 5β-cholestane-3α,7α,12α,26-tetrol into the corresponding carboxylic acid. The extent of conversion varied with different preparations of $LADH_{SS}$ between 0.2 and 0.8 μmoles/min/mg protein when using a glycine buffer, pH 10.0. The reaction was linear with enzyme up to about 4 μg of $LADH_{SS}$ and with time for at least 20 min. The enzyme was saturated with substrate and the apparent K_m was about 5×10^{-5}M.

3β-Hydroxysteroid dehydrogenase activity.

$LADH_{SS}$ catalyzed oxidation of 3β-hydroxy-5β-androstan-17-one into 5β-androstane-3, 17-dione at a rate of about 1.5-2.0 μmoles/min/mg protein. The reaction was linear with enzyme up to at least 2 μg of $LADH_{SS}$ and with time for at least 20 min. The enzyme was saturated with substrate and the K_m was calculated to be 1×10^{-5}M. The reaction was inhibited by 10 mM ethanol by about 50%.

The rate of oxidation of all substrates was reduced by about 90% when the native $LADH_{SS}$ was replaced by carboxymethylated $LADH_{SS}$.

Comparison between $LADH_{SS}$ and $LADH_{EE}$ catalyzed activities.

$LADH_{SS}$ and $LADH_{EE}$ freshly prepared from horse liver (6) were used in parallel experiments designed to compare the different activities. $LADH_{SS}$ was found to have more than fifty times higher ω-hydroxysteroid dehydrogenase activity and about twice higher ω-hydroxyfatty acid dehydrogenase activity than $LADH_{EE}$ (cf. above and ref. 5). The ethanol dehydrogenase activity of $LADH_{SS}$, however, was only about one-third of that of $LADH_{EE}$. The $LADH_{EE}$ had no detectable 3β-hydroxysteroid dehydrogenase activity. The ethanol concentration needed to inhibit ω2-hydroxyfatty acid dehydrogenase by 50% was more than ten times higher with $LADH_{SS}$ than with $LADH_{EE}$ (cf. above and ref. 5).

DISCUSSION

The present work shows that $LADH_{SS}$ possessed ω-hydroxy-fatty acid dehydrogenase activity and ω-hydroxysteroid dehydrogenase activity. A specific carboxymethylation of a cysteine residue at the active site was followed by loss of ω-hydroxy-fatty acid dehydrogenase activity as well as ω-hydroxysteroid dehydrogenase activity and ethanol dehydrogenase activity, showing that $LADH_{SS}$ itself and not contaminating enzymes is responsible for the activities studied. The rate of oxidation of ω-hydroxyfatty acids by $LADH_{EE}$ and $LADH_{SS}$ was of similar magnitude (cf. ref. 5). The stereochemistry of oxidation of ω2-hydroxyfatty acids by $LADH_{SS}$ and $LADH_{EE}$ was also similar; in both cases, the rate of oxidation of the 17-L-enantiomer of 17-hydroxystearic acid was considerably faster than that of the 17-D-enantiomer (cf. ref. 5). It is noteworthy that a higher

concentration of ethanol was needed to inhibit ω2-hydroxyfatty acid dehydrogenase activity of $LADH_{SS}$ than of $LADH_{EE}$ (cf. ref. 5). The capacity of $LADH_{SS}$ to catalyze oxidation of a hydroxyl group in the side chain of a C_{27}-steroid was considerably greater than that of $LADH_{EE}$.

In addition, $LADH_{SS}$ but not $LADH_{EE}$ was active towards a 3β-hydroxyl group at the opposite end of the steroid nucleus, indicating alternative binding positions for bulky steroid substrates. A large hydrophobic substrate pocket, with binding properties different from that of $LADH_{EE}$ is therefore suggested at the active site of $LADH_{SS}$.

ACKNOWLEDGEMENTS

This work has been supported by the Swedish Medical Research Council (Projects 13X-3141, 13X-3532, 13X-2195) and by a grant from NIAAA, USA (R501-AA00323-02). The skilful technical assistance of Miss Eva Strindberg is gratefully acknowledged.

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