Purification and Partial Characterization of 3-Hydroxyisobutyryl-coenzyme A Hydrolase of Rat Liver*

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An unusual feature of valine catabolism is a reaction in which an intermediate of its catabolic pathway, (S)-3-hydroxyisobutyryl-CoA, is hydrolyzed to give the free acid and CoA-SH. The enzyme responsible for this reaction, 3-hydroxyisobutyryl-CoA hydrolase (EC 3.1.2.4), was purified 7200-fold from rat liver in this study. The purified enzyme consists of a single polypeptide with an M, of 36,000 in the native and denatured forms. The hydrolase is highly specific for (S)-3-hydroxyisobutyryl-CoA and 3-hydroxypropionyl-CoA (Km = 6 and 25 µM, respectively) with optimal activity around pH 8. The turnover rate of the enzyme for (S)-3-hydroxyisobutyryl-CoA is 270 s⁻¹, which is higher than that of other enzymes of the valine pathway. Likewise, activity of the enzyme expressed in a wet weight basis is also very high in the major tissues of the rat. These findings suggest that rapid destruction of (S)-3-hydroxyisobutyryl-CoA produced during valine catabolism is physiologically important. We propose that the need for a mechanism to protect cells against the toxic effects of methacrylyl-CoA, which is maintained in equilibrium with (S)-3-hydroxyisobutyryl-CoA by crotonase, explains why valine catabolism involves this enzyme and why its tissue activity is so high.

3-Hydroxyisobutyryl-CoA (HIB-CoA) hydrolase (3-hydroxy-2-methylpropionyl-CoA hydrolase, EC 3.1.2.4) is responsible for the hydrolysis of (S)-HIB-CoA (1), an intermediate in the pathway of valine catabolism. This enzyme was partially purified from pig heart and some of its properties were reported in 1957 by Rendina and Coon (1). Purification of HIB-CoA hydrolase to homogeneity as well as further characterization of some of its properties are reported here. The enzyme is of particular interest from the standpoint of why the reaction it catalyzes should even occur in cells. A monocarboxylic acid is produced (R,S)-3-hydroxyisobutyric acid) that readily diffuses out of cells in which it is formed. Indeed, it seems paradoxical that an acyl-CoA hydrolase should be required for this pathway when both proximal and distal parts of the pathway involve CoA ester intermediates. Furthermore, HIB-CoA hydrolase must be very specific for its substrate to avoid interference with catabolism of fatty acids, leucine, and isoleucine. Although there has been much interest in the interorgan traffic of (S)-3-hydroxyisobutyrate and its possible role as a substrate for various processes (2-6), we propose here that the reason for hydrolysis of (S)-HIB-CoA is to protect cells against toxic effects of methacrylyl-CoA, an intermediate in the valine pathway occurring upstream of (S)-3-HIB-CoA.

EXPERIMENTAL PROCEDURES

Materials—For enzyme purification, livers were obtained from decapitated Sprague-Dawley rats that had been bred and raised in the laboratory. The tissue was stockpiled at -80 °C until sufficient quantities had accumulated. For tissue distribution studies, male Wistar rats were obtained from Harlan Industries (Indianapolis). Liver mitochondria were prepared by the method of Johnson and Lardy (7). Methacrylic anhydride, methacryloyl chloride, acryloyl chloride, and the S- and R-isomers of methyl-3-hydroxyisobutyrate were purchased from Sigma or Kojin Co., Ltd., Tokyo; crotonase, glutathione, glutathione-agarose, and thrombin were purchased from Sigma. Methacrylyl-CoA and acrylyl-CoA were prepared from methacrylic anhydro derivative (methacryloyl chloride) and acryloyl chloride, respectively, and CoA-SH according to the method of Stern and Campbell (8) for the synthesis of crotonyl-CoA. Purification was accomplished by DEAE-cellulose chromatography as described by Lau et al. (9). (S)- and (R)-HIB-CoA were synthesized from the corresponding acids (obtained by alkaline hydrolysis of the (S)- and (R)-methyl esters) according to the method of Wieland and Rueff (10). Purification was accomplished by reverse-phase high-performance liquid chromatography using an Applied Biosystems 130A separation system (220 × 2.1-ram C18 microbore column; 0→30% acetonitrile gradient). Both (R)- and (S)-HIB-CoA eluted as single peaks at 12.7% acetonitrile, gave single spots (Rf = 0.69) on thin layer chromatography (Avicel plates, H2O-saturated butanol as the solvent system). The following CoA esters were obtained from Sigma: acetoacetyl-, acetyl- benzoyl-, n-butyryl-, crotonyl-, crotonyl, glutaryl-, n-3-hydroxybutyryl-, 3-hydroxy-3-methylglutaryl-, isobutryl-, isovaleryl-, malonyl-, 3-methylcrotonyl, n-methylmalonyl-, palmitoyl-, phenylacetyl-, propionyl-, succinyl-, tiglyl-, and n-valeryl-. (R)- and (S)-ibuprofenyl-CoA were kindly gifts from Dr. Stephen D. Hall (Department of Medicine, Indiana University School of Medicine, Indianapolis, IN). Phenyl-Serapho1 CL-4B, octyl-Sepharose CL-4B, DEAE-Sephacel, CM-Sepharose CL-6B, Sepharose S-200 HR, and thiopropyl-Sepharose 6B were purchased from Pharmacia LKB Biotechnology Inc.; hydroxylapatite (Bio-Gel HTP) was purchased from Bio-Rad; and ultrafiltration membrane YM-10 was purchased from Amicon, Inc. CoA-Sepharose was prepared from CoA-SH and thiopropyl-Sepharose 6B according to the instructions provided by Pharmacia. Recombinant 3-hydroxyisobutyrate dehy-
dorgenase, expressed as a glutathione S-transferase fusion protein in Escherichia coli JM109 harboring a 3-hydroxyisobutyrate dehydrogenase expression vector, was purified to homogeneity by glutathione-agarose affinity chromatography. The fusion protein was cleaved with thrombin and the active enzyme used for the quantitative assay of 3-hydroxyisobutyrate dehydrogenase.

**Composition of Buffers**—Buffer A consisted of 50 mM potassium phosphate, pH 7.5, containing 0.1 mM EDTA, 0.1 μM leupeptin, and 10 μg/ml trypsin inhibitor; Buffer B was 25 mM potassium phosphate, pH 7.5, containing 0.1 mM EDTA.

**Purification Procedures**—All procedures were performed at 0–4°C. Frozen livers in 180-g portions were homogenized with an Oster blender (household type) at full speed for 4 min in 720 ml of Buffer A containing 1 mM EDTA, 1% bovine serum, 10 μM N-tosyl-l-phenylalanine chloromethyl ketone, 1 μM phenylmethylsulfonyl fluoride, and 0.5% Triton X-100. The homogenate was centrifuged at 9500 x g for 15 min. The supernatant was passed through four layers of cheesecloth, and the pH was adjusted to 7.5 with 2.5 M Tris.

The supernatant was made to 30% saturation in ammonium sulfate (176 g/liter) by slow addition of a solid salt with constant stirring and was allowed to stir for 20 min before centrifugation at 9500 x g for 20 min. The pellet was resuspended in Buffer A and dialyzed against 10 mM Tris-C1, pH 8.5, containing 0.1 mM EDTA and 0.1 M NaCl. The column was further eluted with the same buffer, and the pH was adjusted to 7.5 with 0.1 M NaCl. The column was further eluted with the same buffer containing 0.5 M NaCl to remove proteins bound to the column and equilibrated again with 10 mM Tris/citrate buffer, pH 5.6, containing 0.1 mM EDTA and 0.05% Tween 20. Fractions with the hydrolase activity eluted with the buffer containing 0.1 M NaCl were combined. The NaCl concentration was decreased to less than 10 mM by ultrafiltration method, and the sample was applied again to the CoA-Sepharose column. The column was washed with 10 ml of the equilibrating buffer and eluted with Buffer B (pH 7.5) containing 0.05% Tween 20.

**Assay of Hydrolase Activity**—The hydrolase activity was assayed spectrophotometrically at 30°C in a total volume of 1 ml with 0.1 M Tris-HCl, pH 8.0, containing 1 mM EDTA, 0.01% Triton X-100, 0.1 mM DTNB, 0.2 mM methacrylyl-CoA, and 10 units of crotonase. Approximately 1 min after addition of methacrylyl-CoA and crotonase, the reaction was started by addition of the indicated amount of the hydrolase dissolved in Buffer A containing 0.1% Tween 20, and the absorbance change at 412 nm due to the reduction of DTNB (11) was monitored. The 3-hydroxypropionyl-CoA hydrolase assay was assayed in the same manner except that acrylyl-CoA was used in place of methacrylyl-CoA. One unit of the hydrolase catalyzed the formation of 1 pmol of CoA-SH/min.

**Assay of Hydrolase Activity at Various pH Values**—A reaction mixture in a total volume of 1 ml with buffer (30 mM Mes, 30 mM Heps, 30 mM Taps, 30 mM CAPS) containing 50 mM NaCl, 1 mM EDTA, 0.1% Triton X-100, 0.2 mM methacrylyl-CoA, and 10 units of crotonase was used after adjustment to indicated pH with NaOH at 30°C. Approximately 1 min after addition of methacrylyl-CoA and crotonase, the reaction was started by the addition of 0.015 μl of the hydrolase followed by incubation at 30°C for 2.5 min. The reaction was stopped by the addition of 0.1 ml of a mixture of 18% trichloroacetic acid and 1% SDS. The pH of the mixture was adjusted to ~5 by the addition of 1.6 M Tris-HCl, pH 8. Then, 0.1 ml of 1 M DTNB was added and absorbance at 412 nm was measured. A control mixture without the hydrolase was prepared and treated in the same manner. The absorbance obtained after enzyme reaction was corrected by the absorbance of control reagent.

In the case of methylnomalonic-CoA (0.3 μM) used as substrate, crotonase was omitted from the reaction mixture, 0.54 μg of the hydrolase was used, and the incubation for the reaction was performed at 30°C for 40 min.

**Analytical Methods**—3-Hydroxyisobutyrate was quantitated by the method of Rougraff et al. (12) with recombinant 3-hydroxyisobutyrate dehydrogenase as the enzyme source. SDS-PAGE was performed as described by Laemmli (13) except the acrylamide concentration was 12%. Samples for electrophoresis were treated as described previously (14). Protein determination was by the BCA method with bovine serum albumin as standard (15).

**RESULTS**

**Purification of HIB-CoA Hydrolase from Rat Liver**—HIB-CoA hydrolase was purified 7200-fold with an overall yield of 3.6% (Table 1). Hydroxypatite column chromatography proved to be one of the most effective steps of the purification with hydrolase activity being eluted with 75 mM phosphate buffer immediately after a major protein peak (Fig. 1). In the first Sephacryl S-200 column chromatography step (Fig. 2), it was found important to combine and recover only fractions with very high ratios of hydrolase activity to absorbance at 280 nm. Tween 20 had to be added to all buffers after hydroxypatite column chromatography to minimize loss of the enzyme by adsorption to plastic. The final preparation consisted of a single

2. J. W. Hawes and R. A. Harris, unpublished method.
3-Hydroxyisobutyryl-CoA Hydrolase

Table I

| Purification step | Protein | Total activity | Yield | Specific activity |
|------------------|---------|---------------|-------|------------------|
| Crude homogenate* | 62,700  | 3,480         | 100   | 0.056            |
| (NH₄)₂SO₄ (45-75%) precipitate | 12,900  | 2,451         | 70    | 0.19             |
| Phenyl-Sepharose and (NH₄)₂SO₄ precipitate | 4,073   | 1,896         | 54    | 0.47             |
| Octyl-Sepharose and (NH₄)₂SO₄ precipitate | 2,934   | 1,828         | 53    | 0.62             |
| DEAE-Sephalocel eluate | 500     | 864           | 25    | 1.73             |
| CM-Sepharose eluate | 82.1    | 522           | 15    | 6.36             |
| Hydroxylapatite eluate | 6.61    | 360           | 10    | 55.4             |
| DEAEXephacel eluate | 500     | 864           | 25    | 1.73             |
| Sphacryl S-200 eluate | 0.85    | 188           | 5.4   | 222              |
| CoA-Sepharose eluate | 0.40    | 161           | 4.6   | 403              |
| Sphacryl S-200 eluate | 0.29    | 126           | 3.6   | 427              |

* Refers to protein and activity of supernatant after centrifugation.

The following salts and nucleotides were without effect upon HIB-CoA hydrolase activity: 60 mM KCl and NaCl; 5 mM CaCl₂ and MgCl₂; and 3 mM ATP, ADP, NAD⁺, and NADH.

Substrate Specificity and Kinetics—(S)-HIB-CoA, produced from methacryl-CoA by the action of crotonase, was the best substrate for HIB-CoA hydrolase under standard conditions (Table II). 3-Hydroxypropionyl-CoA, produced from acryl-CoA by the action of crotonase, was also a good substrate. Ten other CoA esters listed in Table II were hydrolyzed by the enzyme but at rates all less than 1% of (S)-HIB-CoA. (R)-HIB-CoA was not hydrolyzed by the enzyme, indicating HIB-CoA hydrolase is stereospecific for the S-isomer. Hydrolysis of the following compounds by the enzyme also could not be detected: acryl-, benzoyl-, n-butyryl-, crotonyl-, glutaryl-, 3-hydroxyisovaleryl- (produced from 3-methylcrotonyl-CoA by crotonase), 3-hydroxy-3-methylglutaryl-, (R)-ibuprofenyl-, (S)-ibuprofenyl, isovaleryl-, 3-methylcrotonyl-, methacryl-, palmitoyl-, phenylacetyl-, succinyl-, and tiglyl-CoA. Thus, HIB-CoA hydrolase is highly specific for the hydrolysis of (S)-HIB-CoA and 3-hydroxypropionyl-
CoA. That the end products of the reaction catalyzed by HIB-CoA hydrolase with methacrylyl-CoA (plus crotonase) as substrate were indeed (S)-3-hydroxyisobutyrate and CoA-SH was confirmed by quantitating the amounts of each produced under standard assay conditions. The ratio of (S)-3-hydroxyisobutyrate to CoA-SH produced was 1.0 ± 0.1 (mean ± S.D. for three separate determinations).

For kinetic analysis, initial concentrations of (S)-HIB-CoA and 3-hydroxypropionyl-CoA were calculated from the extents of their formation from methacrylyl-CoA and acrylyl-CoA at equilibrium by the hydration reactions catalyzed by crotonase. According to the optical assay described previously (17), 37% of methacrylyl-CoA and nearly 100% of acrylyl-CoA were converted to their respective hydroxy-CoA esters within seconds under the standard conditions of the HIB-CoA hydrolase assay. Lineweaver-Burk plots were linear with both substrates with the lowest $K_m$ and turnover number being obtained with (S)-HIB-CoA (Table III).

CoA esters that were hydrolyzed at slow rates inhibited (S)-HIB-CoA hydrolisis competitively (Table II). $K_i$ values were relatively high compared with the $K_m$ value for (S)-HIB-CoA.

![Figure 4](image)

**Figure 4.** pH activity profiles of HIB-CoA hydrolase with (S)-HIB-CoA (○) and methylmalonyl-CoA (□) as substrates. The assay conditions of enzyme activity are described under "Experimental Procedures."

**Table II**

Substrate specificity of HIB-CoA hydrolase and inhibition constants for various CoA esters

| CoA esters                  | Activity | Inhibition |
|-----------------------------|----------|-----------|
|                            | units/mg | $K_i$ (mM) Type |
| Methacryl-CoA + crotonase   | 430a     | 100       |
| (S)-HIB-CoA                 |          |           |
| Acryl-CoA + crotonase       | 244      | 57.1      |
| (3-hydroxypropionyl-CoA)    |          |           |
| Tiglyl-CoA + crotonase      | 3.14     | 0.73      |
| (3-hydroxy-2-methylbutyryl-CoA) | 0.77 | |
| Crotonyl-CoA + crotonase    | 1.75     | 0.41      |
| (L-3-hydroxybutyryl-CoA)    |          |           |
| n-3-Hydroxybutyryl-CoA      | 1.15     | 0.27      |
| Acetoacetyl-CoA             | 0.94     | 0.22      |
| n-Methylmalonyl-CoA         | 0.64     | 0.15      |
| Isobutyryl-CoA              | 0.34     | 0.08      |
| Malonyl-CoA                 | 0.26     | 0.06      |
| Acetyl-CoA                  | 0.17     | 0.04      |
| Propionyl-CoA               | 0.17     | 0.02      |
| n-Valeryl-CoA               | 0.09     | 0.02      |

* The enzyme activity was assayed under standard conditions except that the concentrations of CoA esters were at 0.5 mM, and crotonase was omitted for CoA esters other than those indicated. For (S)-HIB-CoA and 3-hydroxypropionyl-CoA, 0.011 μg of enzyme protein was used, and for the other CoA esters 0.34 μg of enzyme protein was used.

+ C, competitive. The values for $K_i$ and type of inhibition were determined by the method of Dixon and Webb (16).

+ Acetoacetyl-CoA was used at a concentration of 0.2 mM.

**DISCUSSION**

HIB-CoA hydrolase has been purified to homogeneity from rat liver. The purified enzyme exhibits very high activity with (S)-HIB-CoA and 3-hydroxypropionyl-CoA as substrates. Activity was detectable with several short-chain CoA esters, but 1-HIB-CoA is not a substrate. No cofactors are necessary for enzyme activity. The enzyme exists as a monomer with a molecular weight of 36,000.

HIB-CoA hydrolase had been previously purified 8-fold from an alcohol-KCl extract of pig heart (1). A much lower pH optimum for enzyme activity was reported for the partially purified enzyme (1) than found in the present study with the purified enzyme. The reason for the apparent discrepancy is not known, but a pH optimum slightly on the alkaline side makes good physiological sense considering the intracellular location of HIB-CoA hydrolase (mitochondrial matrix space).

HIB-CoA hydrolase shows great substrate specificity for (S)-HIB-CoA and 3-hydroxypropionyl-CoA, thereby restricting its action to the valine catabolic pathway and a minor pathway for propionate catabolism involving the latter CoA ester. The degree of specificity exhibited by the enzyme undoubtedly is important in preventing interference with numerous metabolic pathways.
processes involving CoA ester intermediates. Although the enzyme was found to hydrolyze nine additional CoA esters, all were poor substrates relative to (S)-HIB-CoA. It is not likely that HIB-CoA hydrolase can hydrolyze any of these CoA esters effectively under physiological conditions. However, their hydrolysis may be catalyzed in the event that their concentration became markedly elevated due to a defect in a downstream process involving CoA ester intermediates. Although the enzyme was found to hydrolyze nine additional CoA esters, all were poor substrates relative to (S)-HIB-CoA.

The characteristics of the enzyme described by Kovachy et al. (18, 19) are quite similar to those of HIB-CoA hydrolase (18). The enzyme was found to hydrolyze nine additional CoA esters, all of which were poor substrates relative to (S)-HIB-CoA.

The enzyme was found to hydrolyze nine additional CoA esters, all of which were poor substrates relative to (S)-HIB-CoA.

Hydrolysis of 2-methyl-3-hydroxybutyryl-CoA by HIB-CoA hydrolase may occur under some conditions. This CoA ester is formed by crotonase from tiglyl-CoA, an intermediate in the propanoyl-CoA pathway by an enzyme defect. Since it seems that (S)-HIB-CoA could be converted to (S)-methylmalonyl-CoA in just two steps (by an alcohol dehydrogenase followed by an aldehyde dehydrogenase as depicted by dashed lines in Fig. 5) rather than the four steps actually used by the pathway, including one that requires ATP, it seems odd that nature chose to sacrifice a CoA ester in the middle of what could have been a much simpler pathway.

It is interesting to consider why a step is included in the valine catabolic pathway (Fig. 5) in which a CoA ester is hydrolyzed to a free carboxylic acid. Destruction of an activated acyl group is rare in metabolic pathways, particularly when subsequent steps of the pathway also involve activated intermediates. Since it seems that (S)-HIB-CoA could be converted to (S)-methylmalonyl-CoA in just two steps (by an alcohol dehydrogenase followed by an aldehyde dehydrogenase as depicted by dashed lines in Fig. 5) rather than the four steps actually used by the pathway, including one that requires ATP, it seems odd that nature chose to sacrifice a CoA ester in the middle of what could have been a much simpler pathway. However, hydrolysis of (S)-HIB-CoA may be an important strategy for disposal of methacrylyl-CoA by cells. The latter compound is a thiol-reactive molecule that undoubtedly would inactivate numerous enzymes in the absence of a mechanism designed to minimize its intramitochondrial concentration (22, 23). In a simpler pathway involving two dehydrogenases for direct conversion of (S)-HIB-CoA to (S)-methylmalonyl-CoA, the methacrylyl-CoA concentration would likely vary with the mitochondrial redox state, perhaps allowing toxic concentrations of this thiol-reactive CoA ester to accumulate under conditions of reducing equivalent overload.

The clinical experience with an infant born with an almost complete deficiency of HIB-CoA hydrolase lends credence to the

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**Table III**

| Kinetic characterization of HIB-CoA hydrolase |
|-----------------|-----------------|
| Constant        | Substrate       |
| (S)-HIB-CoA     | 3-Hydroxypropionyl-CoA |
| $V_{max}$ (units/mg) | $K_m$ (mM) | Turnover number (s⁻¹) |
| 443 ± 3         | 6.0 ± 0.2       | 266               |

* Values were calculated from approximately 37 and 100% conversion of methacrylyl-CoA to (S)-HIB-CoA and acrylyl-CoA to 3-hydroxypropionyl-CoA, respectively, at equilibrium in the presence of crotonase.

**Table IV**

| Distribution of HIB-CoA hydrolase in rat tissues |
|-----------------|
| Tissue          | Activity* |
| Liver            | 9.9 ± 0.8 |
| Kidney           | 6.2 ± 0.3 |
| Heart            | 8.3 ± 0.3 |
| Muscle           | 2.0 ± 0.4 |
| Brain            | 2.7 ± 0.1 |

* HIB-CoA hydrolase activity was assayed under standard conditions. Each value is the mean ± S.E. for tissues from three animals.

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**Fig. 5. Valine catabolic pathway.** HIBDH, 3-hydroxyisobutyrate dehydrogenase; MMSDH, methylmalonate semialdehyde dehydrogenase. Dashed lines indicate hypothetical pathways.
above interpretation (22). The child exhibited multiple congenital physical malformations, suggesting that a defect in this enzyme may be teratogenic. Death from a cardiac lesion occurred at 3 months. During life, the patient excreted large amounts of cysteine/cysteamine conjugates of methacrylic acid, indicating that conjugation between methacrylyl-CoA and glutathione occurred. Methacrylyl-CoA (but not the free acid) reacts readily with free thiol groups of proteins (22, 23), suggesting that high concentrations could cause inhibition of enzymes with sensitive sulfhydryl groups. Methacrylate oxygen esters have been reported to be teratogenic (24) and are recognized as genotoxic/clastogenic agents from studies with F344/N rats, B6C3F1 mice, and mouse lymphoma cells (25, 26). Because of the electron-withdrawing carboxylic acid oxygen ester group, compounds such as ethyl acrylate and methyl methacrylate (and the well-known carcinogen acrylamide CH\textsubscript{2}=CHCONH\textsubscript{2}) readily react with the nucleophiles of proteins, DNA, and glutathione (Michael addition). Since thioesters are more electrophilic than oxygen esters (27), we propose that compounds with considerable potential for cytogenic, mutagenic, and clastogenic actions, making it important to maintain their intracellular concentrations extremely low.

Acryl-CoA is a naturally occurring compound produced in small amounts from propionyl-CoA (1). Thus, it is interesting to note that cells use the same strategy to protect against acryl-CoA toxicity as methacryl-CoA toxicity, i.e., conversion of acryl-CoA to 3-hydroxypropionyl-CoA by crotonase followed by thioester cleavage of the latter compound by HIB-CoA hydrolase to give the less reactive compound 3-hydroxypropionate.

In contrast to the relatively low activities and turnover rates for the enzymes in distal and proximal parts of the valine pathway (activity and turnover numbers of 1.2 μmol/min/g, wet weight, of liver and 18 s\textsuperscript{-1} for liver branched chain α-ketoacid dehydrogenase complex (14); 1.0 μmol/min/g, wet weight, and 7 s\textsuperscript{-1} for 3-hydroxyisobutyrate dehydrogenase (28); 0.7 μmol/min/g, wet weight, and 2 s\textsuperscript{-1} for methylmalonate semialdehyde dehydrogenase (29); HIB-CoA hydrolase has markedly higher tissue activity and turnover number (9.9 μmol/min/g, wet weight, and 270 s\textsuperscript{-1}), thereby accomplishing the rapid destruction of (S)-HIB-CoA as well as methacryl-CoA, the latter being due to the reaction catalyzed by crotonase, another enzyme with high tissue activity and turnover number. As a consequence, (S)-HIB-CoA and methacryl-CoA are not detectable in liver cells even when incubated under conditions that should maximize the concentrations of intermediates of the valine pathway (30).

Formation of (S)-3-hydroxyisobutyric acid by the action of HIB-CoA hydrolase produces a carboxylic acid that readily diffuses from its intracellular site of formation. In some tissues, the enzymes that catalyze steps of the valine pathway distal to the reaction catalyzed by HIB-CoA hydrolase are quite poorly expressed, thereby establishing interorgan trafficking of (S)-3-hydroxyisobutyrate (2–6, 21). Although there has been much interest in establishing a physiological role for circulating (S)-3-hydroxyisobutyrate, analogous to the important roles of circulating lactate and ketone bodies, its presence in blood may simply reflect the mechanism that has evolved to minimize methacryl-CoA toxicity.

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