The Conjugation of 2-Aminoethanesulfinic Acid (Hypotaurine) with Cholic Acid by Rat Liver Microsomes

Lorentz Eldjarn and Jon Bremer*

Norsk Hydro's Institute for Cancer Research, Det Norske Radiumhospital, Oslo, Norway

Hypotaurine seems to be the main immediate precursor of taurine in the organism [1]. Taurine is conjugated with cholic acid by an enzyme system in rat liver microsomes [2]. In the present report we have demonstrated that hypotaurine as well is conjugated with cholic acid by this enzyme system, with the formation of "hypotaurocholic acid".

Hypotaurine-^{35}S was synthesized according to Eldjarn, Pihl and Sverdrup [3]. The preparation of rat liver microsomes and the incubation experiments were performed as earlier described [2].

The reaction was stopped and the proteins precipitated by adding 0.15 ml N perchloric acid to the vessels, followed by 0.15 ml N KOH. The reaction mixture was extracted with 0.6 ml *n*-butanol. Aliquots of the butanol extract were plated for radioactivity measurements or chromatographed on paper according to Sjövall [4].

* Fellow of the Norwegian Cancer Society, Oslo.

The paper chromatography showed that mainly taurocholic acid (R_F 0.25) had been formed after incubation of hypotaurine with cholic acid, but in addition a radioactive compound with R_F 0.75 was found. This presumably was hypotaurocholic acid, since no radioactivity was found at this R_F when hypotaurine was incubated without cholic acid. The identity of the unknown compound was confirmed by the following experiment: The radioactive compound at R_F 0.75 was eluted from the paper with ethanol. The eluate was made alkaline with a few drops of N KOH and stored for 24 hours at 37° C. The eluate was then brought to dryness and the residue was extracted twice with 0.1 ml ethanol. The eluate was again chromatographed according to Sjövall [4]. Approximately 60 % of the radioactivity of the unknown compound at R_F 0.75 reappeared at the R_F of taurocholic acid after this treatment, *i. e.* the hypotaurocholic acid had been oxidized to taurocholic acid.

As the sulfinic groups is a less polar group than the sulfonic group, the coefficient:

$$\frac{\text{concentration in butanol phase after extraction}}{\text{concentration in } H_2O \text{ before extraction}}$$

of hypotaurocholic acid presumably is greater than the coefficient of taurocholate which is known [5] to be 2.0. As a coefficient of 3.5 corresponds to quantitative extraction, the coefficient of hypotaurocholate was presumed to be 3.0. From this coefficient, the extractable radioactivity and from the radioactivity on the paper chromatograms the results of Table 1 were calculated.

The table shows that addition of taurine almost completely abolished the formation of

Table 1.

Substrates	Net counts/min/ 0.05 ml butanol extract	µmole taurocholate formed per vessel	µmole hypotaurocholate formed per vessel
0.5 µmole hypotaurine-^{35}S = 340 000 c/min	0	0	0
0.5 µmole hypotaurine-^{35}S + 1 µmole cholate	4 150	0.082	0.019
0.25 µmole hypotaurine-^{35}S = 170 000 c/min + 1 µmole cholate	3 905 3 660	0.075	0.014
0.25 µmole hypotaurine-^{35}S + 2.4 µmoles inactive taurine + 1 µmole cholate	460 400	—	0.001
0.8 µmole taurine-^{35}S = 105 000 c/min + 1 µmole cholate	2 330 2 220	0.307	0
0.8 µmole taurine-^{35}S + 2.4 µmoles inactive hypotaurine + 1 µmole cholate	1 960 1 820	0.275	—

Microsomes from approximately 500 mg rat liver per vessel, CoA 0.001 M, ATP 0.006 M, KF 0.15 M, sucrose 0.05 M, potassium phosphate buffer (pH 7.4) 0.033 M. Gas phase air, incubation time 90 min., temp. 37° C, total volume 1.5 ml.

hypotaurocholate, whereas addition of hypotaurine had no significant effect on the taurocholate formation. Our experiments therefore show that rat liver microsomes can conjugate hypotaurine with cholic acid, but that taurine is preferred as substrate in the reaction. Since rat liver is known to contain great amounts of free taurine [5], the conjugation with hypotaurine can only be of limited physiological importance in the rat.

Whether the conversion of hypotaurine to taurine is enzymatic or spontaneous in our system is not known.

1. Chatagner, F. and Bergeret, B. *Ann. nutrition et aliment.* **9** (1955) 93.
2. Bremer, J. *Acta Chem. Scand.* **10** (1956) 56.
3. Eldjarn, L., Pihl, A. and Sverdrup, A. *J. Biol. Chem. In press.*
4. Sjövall, J. *Acta Chem. Scand.* **8** (1954) 339.
5. Bremer, J. *Acta Chem. Scand.* **9** (1955) 683.

Formation of Itaconic Acid from the Krebs Cycle Tricarboxylic Acids by Extracts of *Aspergillus terreus*

Elizabeth Brigham Jenssen, Helge Larsen and John G. Ormerod

Department of Biochemistry, The Technical University of Norway, Trondheim, Norway

Evidence has been presented that citric acid [1,2] and *cis*-aconitic acid [3] are intermediates and close precursors to itaconic acid in the formation of itaconic acid from sugars by *Aspergillus terreus*.

Cell-free extracts of *A. terreus* can be prepared by crushing rapidly frozen mycelium in the Hughes' bacterial press and extracting the paste with water. Such extracts, when prepared from itaconic acid producing mycelia (*i. e.*, mycelia grown under acid conditions [4]), will catalyze a conversion of citric, *cis*-aconitic and *iso*citric acids to itaconic acid and carbon dioxide. *cis*-Aconitic acid is by far the most readily converted; citric and isocitric acids were in some experiments converted to itaconic acid only to a negligible extent. The optimum pH is about 5.7. Molecular oxygen does not affect the conversions. Extracts prepared from mycelia grown under neutral conditions (*i. e.*, mycelia not producing itaconic acid from sugars [4]), will not catalyze a conversion of the aforementioned tricarboxylic acids to itaconic acid. This finding is in agreement with the previously presented hypothesis that the itaconic acid forming enzyme system is dependent upon acid conditions for its formation [4].

Active extracts decarboxylate 1 mole of *cis*-aconitic acid to give close to 1 mole of carbon dioxide and 1 mole of itaconic acid. Correspondingly, the yield of carbon dioxide from citric acid is close to 1 mole per mole of citric acid converted, whereas the yield of itaconic acid is usually much lower, *viz.* 0.3—0.6 moles per mole of citric acid converted.

Synthetic monofluorocitrate in a concn. of 10^{-4} M causes an appreciable inhibition in the rate of itaconic acid production from citric acid and a slight inhibition in the rate of itaconic acid production from isocitric acid. 10^{-4} M fluorocitrate does not inhibit the rate or lower the yield of itaconic acid formation from *cis*-aconitic acid; there is rather a slight stimulation. Aconitase has been shown to be present in the extract. Assuming the aconitase to be fluorocitrate sensitive [5], the results would indicate that itaconic acid is formed by decarboxylation of *cis*-aconitic acid.

The reversibility of the decarboxylating system has been tested by incubating itaconic acid producing extracts in the presence of *cis*-aconitic acid and $^{14}CO_2$ at pH 5.3. Itaconic acid and residual *cis*-aconitic acid were isolated and tested for radioactivity. Both compounds were found to be non-radioactive. The lack of radioactivity in the *cis*-aconitic acid does not necessarily imply that the conversion is irreversible; it might equally well indicate an extreme slowness in the mixing of the *cis*-aconitic acid supplied and its biologically activated counterpart [6].

The work was supported by grants from the *Royal Norwegian Council for Scientific and Industrial Research*. We are indebted to Professor R. A. Peters for a gift of synthetic tri sodium monofluorocitrate.

1. Corzo, R. H. and Tatum, E. L. *Federation Proc.* **12** (1953) 470.
2. Eimhjellen, K. E. and Larsen, H. *Biochem. J. London* **60** (1955) 139.
3. Bentley, R. and Thiessen, C. P. *Science.* **122** (1955) 330.
4. Larsen, H. and Eimhjellen, K. E. *Biochem. J. London* **60** (1955) 135.
5. Morrison, J. F. and Peters, R. A. *Biochem. J. London* **58** (1954) 473.
6. Swim, H. E. and Krampitz, L. O. *J. Bact.* **67** (1954) 419.