Characterization of the chirality of microbial hydroxycitric acid enantiomers

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Abstract

Hydroxycitric acid (1,2-dihydroxypropane-1,2,3-tricarboxylic acid, HCA) is a phytomolecule, naturally occuring in variety of plants including *Garcinia cambogia* and *Hibiscus subdariffa.* HCA availability is limited by the restricted habitat of plants and difficulty in organic stereoselective synthesis. HCA exists in 4 different isomers only two showing biologically active forms (*2S, 3S*) - HCA and (*2S, 3R*) – HCA Microbes are promising alternative sources for production of large scale stereospecific HCA. Till date only two microbial species are known to produce biologically active form of hydroxycitric acid .Extraction and characterization for its acid and lactone forms have been reported so far in both plants and bacteria.Both isomers are reported for inhibition of enzymes, (*2S, 3S*) - HCA for ATP citrate lyase and (*2S, 3R*) - HCA for pancreatic alpha amylase and alpha glucosidase enzymes. Subsequently both active isoforms have been reported for anti-obesity and anti-diabetic effects respectively. The present work focuses on characterisation of bacterial HCA by studying inhibition patterns with ATP citrate lyase and alpha amylase enzymes.Comparative analysis of inhibition patterns of ATP citrate lyase and alpha-amylase suggest that the microbial HCA produced was (2S-3R) which specifically inhibits alpha-amylase.

Methodology

ATP citrate lyase inhibition

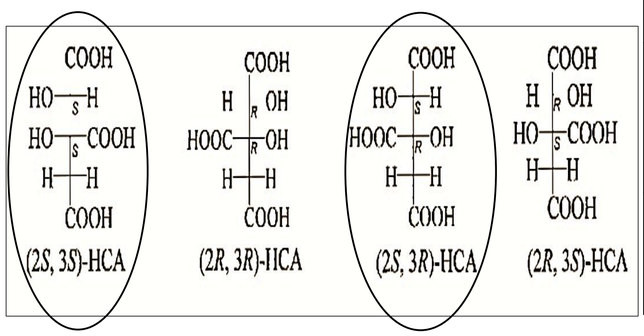
Guinea pig kidney acetone Powder was used as an enzyme source.The assay mixture contained 20 mM of Tris-HCl (pH 6.9), 20mM of trisodium citrate, 10mM of MgCl2, 0.5units of malate dehydrogenase (MDH), 0.1mM of Coenzyme A (CoA), 0.14mM of NADH, 5mM of ATP, and 0.02ml enzyme in a total volume of 1.0ml. Reaction was started by addition of ATP and the rate of NADH oxidation was measured at 340 nm

Alpha amylase inhibition

Starch azure (Sigma Aldrich), 2mg suspended in 0.2ml of 0.5M Tris–HCl buffer (pH= 6.9) containing 0.01M $CaCl_2$ was used as substrate. The reaction was started by adding 0.1ml of porcine pancreatic amylase in Tris–HCl buffer (2U/mL).The reaction was carried out at 37°C for 10 minutes and was stopped by adding 0.5ml of 50% acetic acid in each tube. The reaction mixture was centrifuged at 3000 rpm for 5 min at 4°C and supernatant was used to measure absorbance at 595nm

Introduction

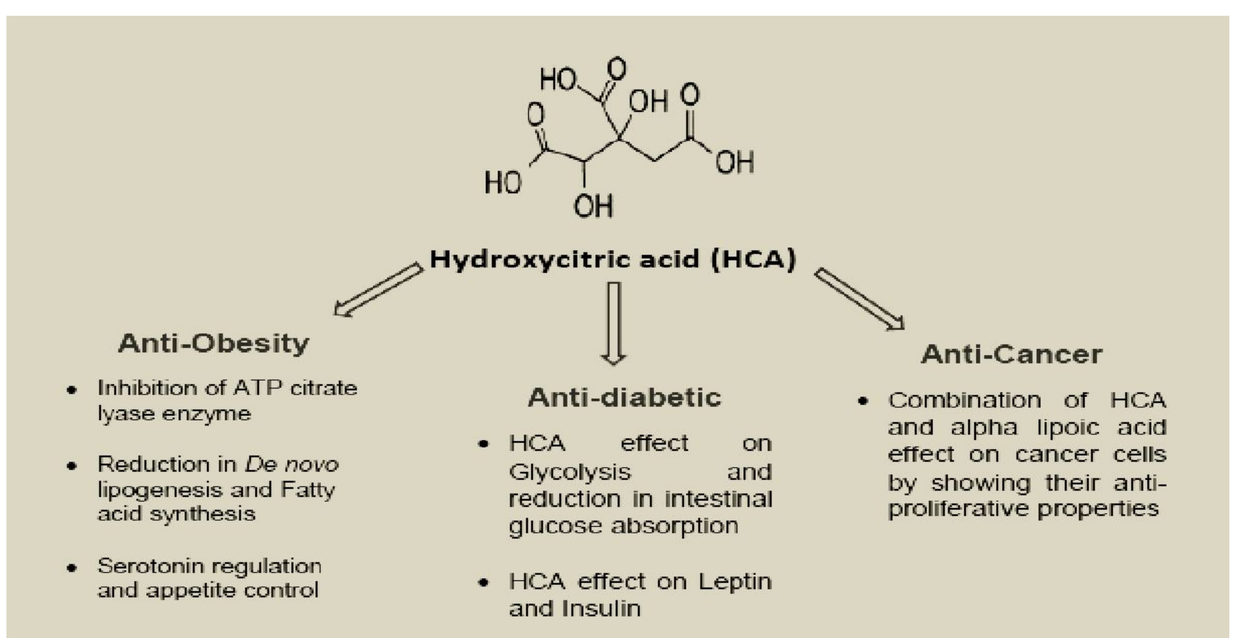
Biologically active isomers of hydroxycitric acid



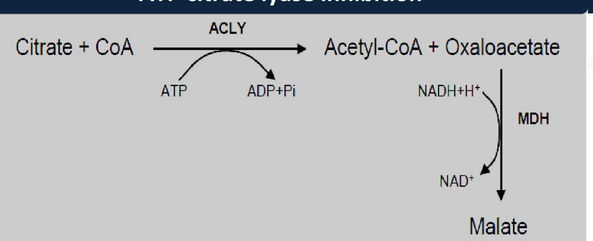
Natural Occurrence of HCA

Plant sources	Bacterial sources
Garcinia combogia *(2S, 3S)*- HCA Anti- Obesity; Anti- cancer	*Bacillus megaterium* G45C **Gram-positive Endospore forming**
Hibiscus subdariffa *(2S, 3R)*- HCA Anti- diabetic	*Streptomyces* spp. U21 **Gram-positive Filamentous organism**

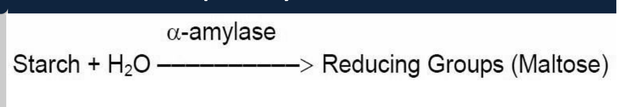
HCA as a therapeutic molecule



ATP citrate lyase inhibition

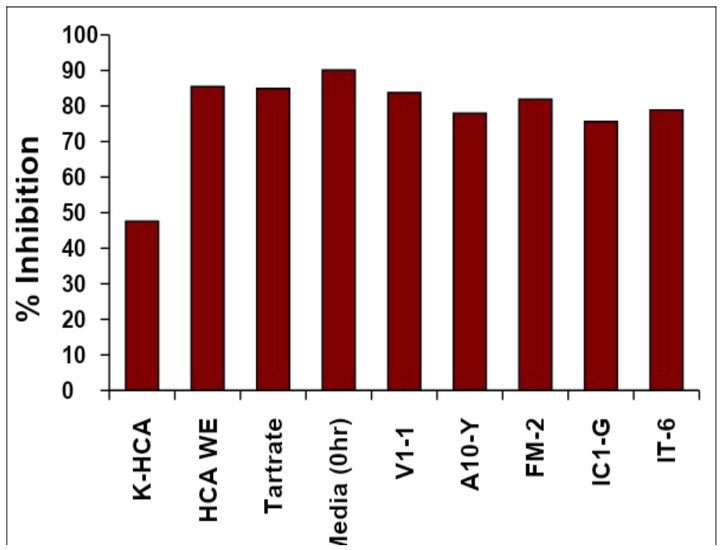


Alpha amylase inhibition



Results and Discussion

HCA stereoselective determination from lyophilized bacterial culture supernatant using ATP citrate lyase (ACL) inhibition

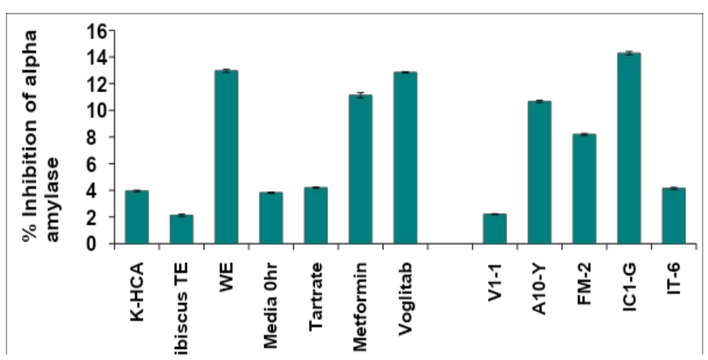


The reaction mixture contained Tris-buffer (pH-6.9), Citrate, ATP and Coenzyme A as substrates, $MgCl_2$ as cofactor (as chloride ions are necessary and could influence ACL activity) and MDH used to measure the rate of NADH oxidation in the presence of inhibitors

- Himalaya Vrikshamla tablet extract and K-HCA (Ki= 150nM) showed 85% inhibition
- 1mM tartrate and 0 h M9 minimal medium showed 85- 90% inhibition
- Hence inhibition observed in cell free culture supernatant of isolates selected cannot be contributed to bacterial HCA

Therefore, overall ACL inhibition pattern remained unclear and was not able to conclude the stereoisomeric nature of bacterial HCA

HCA stereoselective determination from lyophilized bacterial culture supernatant using Alpha-amylase inhibition



- Metformin = 11-12% (Reported inhibition is 10mg/ml)
- Voglitab= 12.08%
- K-HCA and HCA water extract= 4 -12.9%
- Hibiscus tea extract= 2.12%
- Potassium Tartrate(1mM)= 4.19%
- Minimal media at 0hr= 3.8%
- **Bacterial isolates= 2.21 %- 14.28%**

The overall inhibition pattern could suggest that isolates could produce (2S, 3R- HCA). Results were also in accordance with the earlier reports.

References

1. Hida, H., Yamada, T. and Yamada, Y. (2006) 'Absolute configuration of hydroxycitric acid produced by microorganisms', *Bioscience, biotechnology, and biochemistry*,
2. Jena, B.S., Jayaprakasha, G.K., Singh, R.P. and Sakariah, K.K. (2002) 'Chemistry and biochemistry of (−)-hydroxycitric acid from Garcinia' Journal of agricultural and food chemistry, vol. 50, no. 1, January, pp.10-22.

Acknowledgement

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