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A new perspective on the importance of glycine N-acyltransferase in the detoxification of benzoic acid

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Enige iets in die skepping raak interessant as jy mooi genoeg kyk…

Albie van Dijk

Abstract

Despite being the first biochemical reaction to be discovered, the glycine conjugation pathway remains poorly characterised. It has generally been assumed that glycine conjugation serves to increase the water solubility of organic acids, such as benzoic acid and isovaleric acid, in order to facilitate urinary excretion of these compounds. However, it was recently suggested that the conjugation of glycine to benzoate should be viewed as a neuroregulatory process that prevents the accumulation of glycine, a neurotransmitter, to toxic levels. The true importance of glycine conjugation in metabolism is therefore not well understood. However, no genetic defect of glycine conjugation has ever been reported. This seems to suggest that glycine conjugation is a fundamentally important metabolic process, whatever its function may be. Therefore, a major objective of this thesis was to develop a deeper understanding of glycine conjugation and its metabolic significance. A review of the literature on GLYAT and glycine conjugation suggested that the primary purpose of glycine conjugation is indeed to detoxify benzoate and other aromatic acids of dietary origin. However, the commonly held assumption, that glycine conjugation increases the water solubility of aromatic acids in order to facilitate urinary excretion, seems to be incorrect. A better explanation for the detoxification of benzoate by means of glycine conjugation is based on hydrophilicity, not water solubility. Because of its lipophilic nature, benzoic acid is capable of passively diffusing across the mitochondrial inner membrane into the matrix space, where it accumulates due to the pH gradient over the inner membrane. Although benzoate can be exported from the matrix by organic anion transporters, this process would likely be futile because benzoic acid can simply diffuse back into the matrix. Hippurate, however, is significantly less lipophilic and therefore less capable of diffusing into the matrix. It is therefore not transport out of the mitochondrial matrix that is facilitated by glycine conjugation, but rather the ability of the glycine conjugates to re-enter the matrix that is decreased.

The conversion of benzoate to hippurate is a two-step process. First, benzoate is activated by an ATP-dependent acid:CoA ligase (ACSM2A) to form the more reactive benzoyl-CoA. Second, glycine N-acyltransferase (GLYAT) catalyses the formation of hippurate and CoASH from benzoyl-CoA and glycine. Another major objective of this

thesis was to gain a better understanding of the structure and function of the GLYAT enzyme. While the substrate selectivity and enzyme kinetics of GLYAT have been investigated to some extent, almost nothing has been published on the structure, active site, or catalytic mechanism of GLYAT. Furthermore, while interindividual variation in the rate of glycine conjugation has been reported by several researchers, it is not known if, or how, genetic variation in the human GLYAT gene contributes to this interindividual variation. To address these issues, systems for the bacterial expression of recombinant bovine GLYAT and recombinant human GLYAT were developed. Because no crystal structure of GLYAT has been reported, homology modelling was used to generate a molecular model of bovine GLYAT. By comparing the molecular model to other acyltransferases for which the catalytic residues were known, Glu^{227} of bovine GLYAT was identified as a potential catalytic residue. Site directed mutagenesis was used to generate an E227Q mutant recombinant bovine GLYAT lacking the proposed catalytic residue. Characterisation of this mutant suggested that Glu^{227} was indeed the catalytic residue, and the GLYAT catalytic mechanism was elucidated. The molecular model was also used to identify Asn^{131} of bovine GLYAT as a potential active site residue. Site-directed mutagenesis was used to generate an N131C mutant, which was sensitive to inhibition by the sulfhydryl reagent DTNB. This suggests that the Asn^{131} residue of bovine GLYAT may be situated in the active site of bovine GLYAT, but more work is needed to confirm this result. Finally, site-directed mutagenesis was used to generate variants of recombinant human GLYAT corresponding to six of the known SNPs in the human GLYAT gene. Expression and characterisation of the recombinant human GLYAT variants revealed that the enzyme activity and K_M (benzoyl-CoA) parameter of the recombinant human GLYAT were influenced by SNPs in the human GLYAT gene. This suggests that genetic variation in the human GLYAT gene could partly explain the interindividual variation in the rate of glycine conjugation observed in humans. Interestingly, the SNPs that negatively influenced enzyme activity also had low allele frequencies, suggesting that there may be some selective advantage to having high GLYAT activity.

Keywords: Glycine, conjugation, deportation, detoxification, coenzyme A, sequestration, GLYAT, glycine N-acyltransferase, benzoic acid, hippuric acid.

Opsomming

Ten spyte daarvan dat dit die eerste biochemiese reaksie was om ontdek te word, is die glisienkonjugeringsweg steeds nie goed gekarakteriseer nie. Dit is nog altyd aanvaar dat glisien-konjugering die water- oplosbaarheid van organiese sure, soos bensoësuur en isovaleriaansuur, verhoog om uitskeiding in die uriene te versnel. Daar is egter onlangs voorgestel dat die konjugering van glisien aan bensoaat eerder gesien moet word as ’n neuroregulatoriese proses, wat akkumulasie van die neurotransmitter glisien in die brein voorkom. Dus is dit onduidelik wat die ware rol van glisienkonjugering in metabolisme is. Alhoewel die rol van glisienkonjugering onduidelik is, is daar nog nooit ’n genetiese defek van hierdie metaboliese weg beskryf nie. Dit dui daarop dat glisienkonjugering ’n baie belangrike metaboliese proses is. Een van die hoof doelwitte van hierdie tesis was dus om ’n dieper begrip van die glisienkonjugeringsweg, en die rol daarvan in metabolisme, te ontwikkel. Deur ’n deeglike studie van die literatuur te doen, kon die gevolgtrekking gemaak word dat die detoksifisering van bensoaat die hoofdoel van glisien konjugering is. Dit wil voorkom asof die aanname dat glisienkonjugering die wateroplosbaarheid van aromatiese sure verhoog om uitskeiding in die uriene te versnel, verkeerd is. Die ontgifting van bensoaat deur middel van glisienkonjugering berus eerder op ’n verlaging van die lipofilisiteit van die verbinding. Omdat dit ’n lipofiele verbinding is, kan bensoësuur vryelik oor die binne-mitochondriale membraan diffundeer, tot in die mitochondriale matriks, waar dit dan ophoop as gevolg van die pH gradiënt oor die binne-membraan. Alhoewel bensoaat deurmiddel van organiese anioon transporters uit die matriks uitgepomp kan word, sal die proses waarskynlik nutteloos wees. Dit is omdat die bensoaat, in die vorm van bensoësuur, eenvoudig net weer oor die binneste membraan kan diffundeer tot terug in die matriks. Hippuraat is egter ’n baie minder lipofiele verbinding en kan dus nie so maklik terug beweeg oor die binne-mitochondriale membraan nie. Glisienkonjugering detoksifiseer dan nie bensoaat deurdat dit uitvoer vanaf die mitochondriale matriks versnel nie, maar eerder deurdat terugkeer van die uitgeskeide konjugate na die matriks vertraag word.

Bensoaat word omgeskakel na hippuraat in twee stappe. Eers word bensoaat geaktiveer deur ’n ATP-verbruikende asiel-KoA ligase (ACSM2) om die meer reaktiewe bensoïel-KoA te vorm. Daarna kataliseer glisien N-

asieltransferase (GLYAT) die sintese van hippuraat en KoASH vanaf bensoïel-KoA en glisien. Nog ’n hoof doelwit van hierdie tesis was om ’n beter begrip van die struktuur en funksie van GLYAT te bekom. Alhoewel die substraat selektiwiteit en ensiemkinetika van GLYAT al tot ’n mate ondersoek is, is daar nog geen publikasies oor die struktuur, aktiewe setel, of katalitiese meganisme van GLYAT nie. Dit is ook glad nie bekend of variasie in die mens GLYAT geen ’n bydrae maak tot die variasie in glisienkonjugering-snelheid tussen mense nie. Om hierdie probleme aan te spreek, is bakteriële sisteme vir die uitdrukking van rekombinante bees GLYAT en rekombinante mens GLYAT ontwikkel. Omdat daar geen kristal struktuur vir GLYAT bekend is nie, is molekulêre modellering gebruik om ’n model van bees GLYAT te genereer. Deur die bees GLYAT model te vergelyk met ander asieltransferase ensieme met bekende katalitiese meganismes, kon Glu^{227} van bees GLYAT geïdentifiseer word as ’n potensiële katalitiese residu. Deur gebruik te maak van punt-spesifieke mutagenese is ’n E227Q mutant van die rekombinante bees GLYAT, sonder die voorgestelde katalitiese residu, gemaak. Biochemiese karakterisering van die E227Q mutant het die voorstel dat Glu^{227} die katalitiese residu van bees GLYAT is ondersteun, en dus kon ’n katalitiese meganisme vir GLYAT voorgestel word. Die molekulêre model van bees GLYAT is verder ook gebruik om Asn^{131} te identifiseer as ’n residu wat moontlik in die bees GLYAT aktiewe setel voorkom. Punt-spesifieke mutagenese is weer gebruik om ’n N131C mutant te genereer. Hierdie mutant kon geïnhibeer word met die reagens DTNB, wat met die sulfhidriel groep van die N131C mutant reageer. Dit ondersteun die voorstel dat die Asn^{131} residu in die aktiewe setel van bees GLYAT voorkom, maar nog werk moet gedoen word om hierdie resultaat te bevestig. Punt-spesifieke mutagenese is ook gebruik om mutante van ’n rekombinante mens GLYAT te genereer wat ooreenstem met bekende variasies in die mens GLYAT geen. Uitdrukking en karakterisering van hierdie rekombinante mens GLYAT mutante het gewys dat die ensiem aktiwiteit en K_M (bensoïel-KoA) van mens GLYAT beïnvloed word deur variasie in die mens GLYAT geen. Die variasie in glisienkonjugering-snelheid tussen mense kan dus gedeeltelik verklaar word deur genetiese variasie. Omdat die mutante wat ’n negatiewe impak op die ensiemaktiwiteit van mens GLYAT gehad het ook lae alleel frekwensies het, lyk dit asof hoë GLYAT aktiwiteit in die lewer voordelig mag wees.

Sleutelwoorde: Glisien, konjugering, deportasie, detoksikasie, koënsiem A, sekwestrasie, GLYAT, glisien N-asieltransferase, bensoësuur, hippuursuur.

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