3-Hydroxy-3-methylglutaryl-CoA lyase deficiency studied using 2-dimensional proton nuclear magnetic resonance spectroscopy

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^{1}H-NMR spectroscopy has been applied to identify components in the urine of subjects with a deficiency of the enzyme 3-hydroxy-3-methylglutaryl-CoA lyase. One-dimensional spectra of samples from a pair of non-identical twins with this disorder were very similar and are probably diagnostic. The most intense signals were from singlets. Complete assignment of these major components was made possible by the use of 2-dimensional chemical shift correlated spectroscopy since several long-range couplings were detected. 2-dimensional spectroscopic techniques may therefore be of value in the identification of singlets in multicomponent systems.

^{1}H-NMR Metabolism Inborn error 3-Hydroxy-3-methylglutaryl-CoA lyase Enzyme deficiency 2-dimensional NMR

1. INTRODUCTION

The enzyme 3-hydroxy-3-methylglutaryl-CoA lyase (HMG-CoA lyase, EC 4.1.3.4) catalyses the final step of leucine catabolism: the formation of acetyl-CoA and acetoacetate from HMG-CoA (fig.1). HMG-CoA also arises from the condensation of acetyl-CoA and acetoacetyl-CoA and therefore HMG-CoA lyase has a vital role in ketone body formation.

A small number of patients with a deficiency of HMG-CoA lyase have been described [1–4]. These patients characteristically have an abnormally high urinary excretion of metabolites derived from intermediates proximal to the HMG-CoA lyase step (fig.1).

We present here ^{1}H-NMR data of urinary metabolites from two non-identical twins with HMG-CoA lyase deficiency [4]. We also show how application of two-dimensional NMR spectroscopic techniques can aid the identification of spectral peaks and resolution of isomers in urine samples from such patients.

2. MATERIALS AND METHODS

The two patients, a boy and girl, were 5 months old at the time of sampling and have been described in [4]. Urine samples were frozen at −25°C prior to NMR analysis.

Aliquots of urine (0.5 ml) were placed in 5-mm NMR tubes to which were also added 50 μl D_2O and 20 μl 3-trimethylsilyl-2,2,3,3-tetradeuteropropionate (500 mM) for field-locking and as an internal chemical shift reference, respectively. The samples were then run at room temperature in either Bruker AM 360 or WH 400 MHz spectrometers. Usually the sample tube was spun. Solutions of standard compounds (20 mM, pH 6.0) were run under identical conditions. 1-D spectra were collected using either a simple pulse and acquire sequence for recording quantitative data

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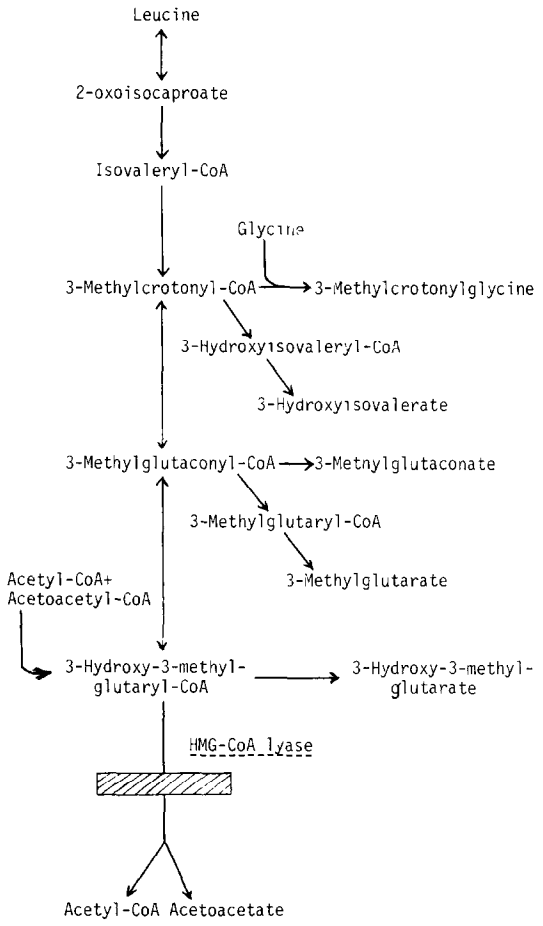


Fig.1. Pathways of L-leucine degradation and ketone body formation showing the block caused by HMG-CoA lyase deficiency. The origin of the major metabolites accumulating in urine is shown.

using 60 pulses every 5 s or a Hahn spin-echo sequence (90-τ-180-τ-acquire) where $\tau = 60$ ms with an interpulse delay of 2 s. 2-D chemical shift correlated NMR spectroscopy (COSY) was used [5] to aid the assignments of the spectra (512 blocks of 16 scans, total time of 272 min). The sweep width was 3300 Hz in both dimensions. The data matrix was ik*ik after transformation and the spectra were symmetrised before display. The water resonance was suppressed by presaturation only during the relaxation delay.

Capillary gas liquid chromatography and mass spectrometry were carried out as described in [4].

3. RESULTS

In ^{1}H-NMR spectra from normal subjects the largest signals arise from the methyl protons of either creatinine (3.04–3.09 ppm) or, in very young children, creatine (3.05 ppm) [6–8]. In contrast to such spectra the 1-D spectra of the patients with HMG-CoA lyase deficiency (fig.2) reveal two large singlets at 1.28 and 1.33 ppm and another pair at 1.88 and 1.96 ppm, a singlet at 2.38 ppm and an AB quartet at 2.47–2.55 ppm. There are also two singlets at 3.0 and 3.45 ppm. In the low-field (aromatic) part of the spectrum (> 5.0 ppm) broad singlets are present at 5.74 and 5.82 ppm. On the basis of spectra of standard solutions these resonances could be assigned to 3-hydroxyisovalerate [1.28 (CH_3) and 2.38 (CH_2) ppm] and 3-hydroxy-3-methylglutarate [1.33 (CH_3) and AB quartet (CH_2) 2.47–2.55 ppm]. There is also a small doublet at 0.93 ppm which is probably from the CH_3 of 3-methylglutarate [multiplets at 2.03 (CH_2, ABX + CH) and 2.27 (CH_2, ABX) ppm].

The 2-D chemical shift correlated spectrum reveals the presence of several 'long-range' couplings between the singlets at 1.28 and 2.38 ppm, the singlet at 1.33 and AB quartet at 2.47–2.55 ppm, singlets at 1.88 and 5.82 ppm, 1.96 and 5.74 ppm, and between singlets at 3.0 and 5.74 ppm. The ratio of the peak areas of the singlets at 1.88, 3.45 and 5.85 was approx. 3:2:1, a similar ratio was found for the singlets at 1.96, 3.0 and 5.74. The presence of long-range coupling constants, the value of the above ratios and the relative chemical shifts suggested that each group of resonances originated from a compound of the type:

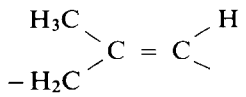
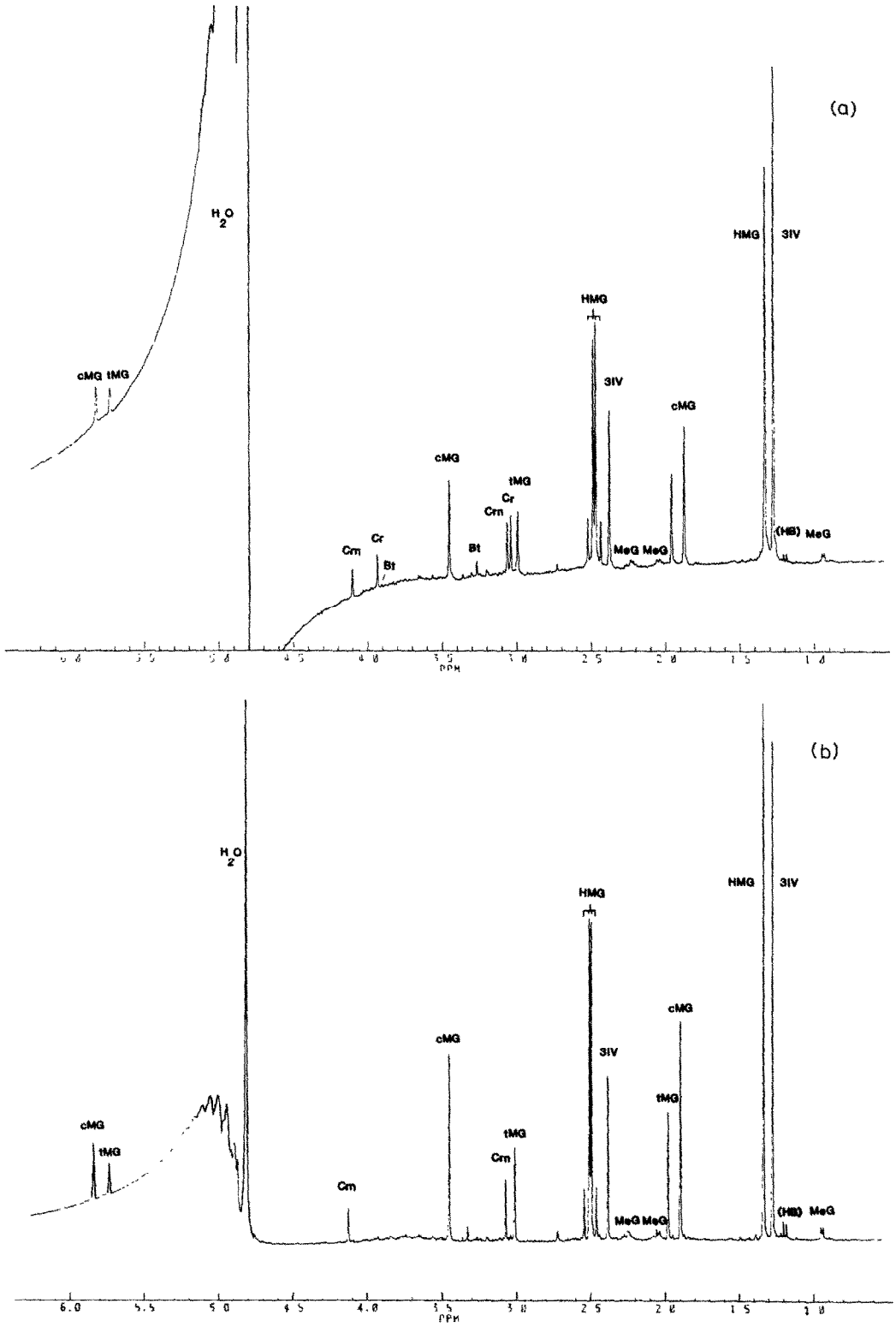


Fig.2. ^{1}H-NMR spectra of urine from (a) the female patient and (b) her twin brother, who both have HMG-CoA lyase deficiency [4]. Bt, betaine; Cr, creatine; Crn, creatinine; 3IV, 3-hydroxyisovalerate; HMG, 3-hydroxy-3-methylglutarate; MeG, 3-methylglutarate; cMG, *cis*-3-methylglutaconate; tMG, *trans*-3-methylglutaconate; (HB), 3-hydroxybutyrate (tentative assignment).


(a)
H2O
cMG tMG
cMG
tMG
Cr
Crn
Cm
Cr
Bt
HMG
3IV
cMG
HMG 3IV
(HB) MeG
MeG MeG
PPM
(b)
H2O
cMG tMG
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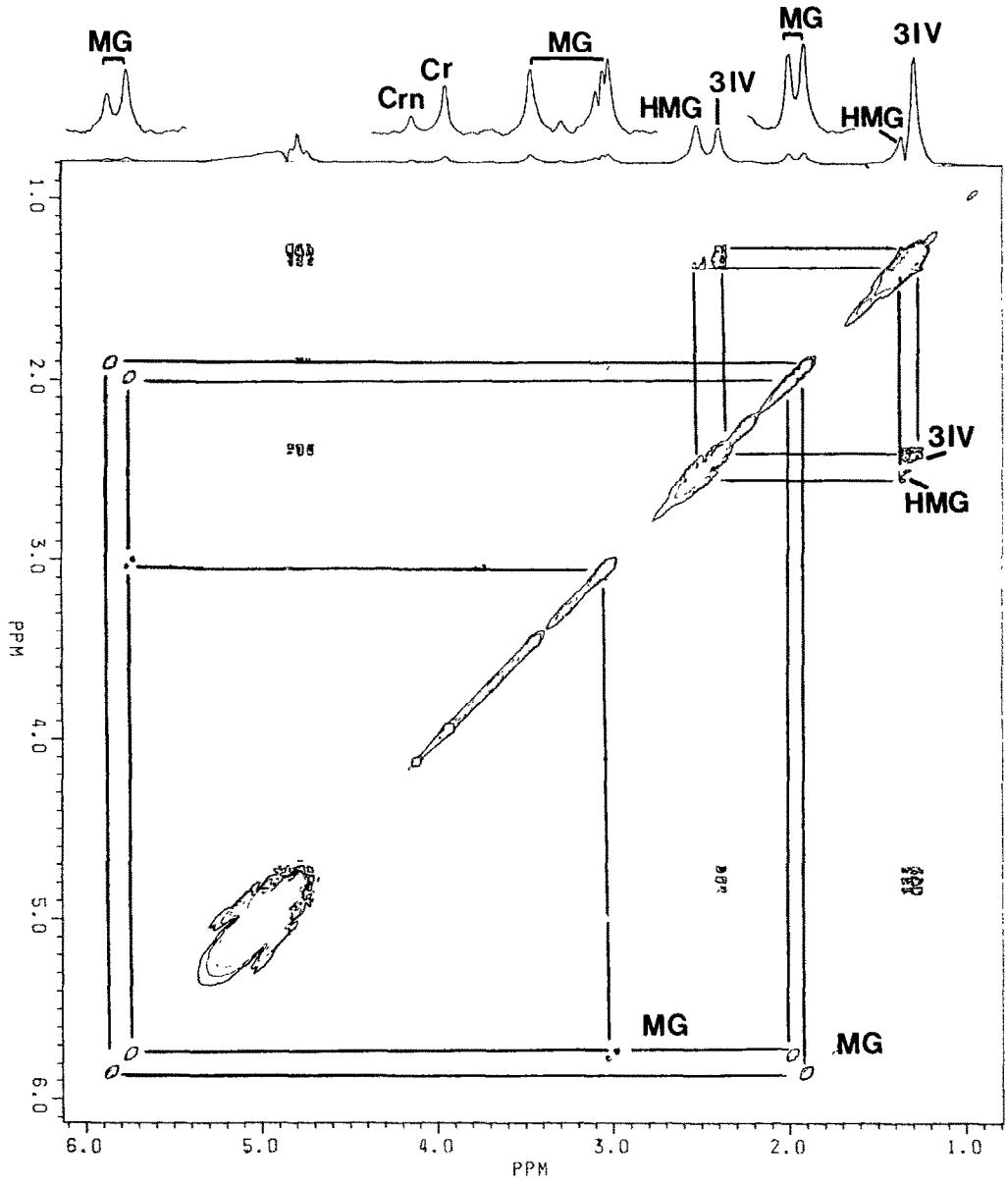

Fig.3. 2-D chemical shift correlated (COSY) ^{1}H-NMR spectrum of urine from the male subject with 3-hydroxy-3-methylglutaric aciduria. MG, 3-methylglutaconate. Other abbreviations are as for fig.2.

It therefore seemed likely that the 3 pairs of coupled resonances were from the *cis* and *trans* isomers of 3-methylglutaconate for which an authentic standard was not available but which is known to accumulate in HMG-CoA lyase deficiency [1,2]. By analogy with the relative chemical shifts of the geometric isomers of methyl crotonate quoted by Emsley et al. [9] the resonances at 1.88, 3.45 and 5.82 ppm would be from the *cis* isomer and those at 1.96, 3.0 and 5.74 ppm from the *trans* isomer (fig.2). The *cis-trans* ratio would be approx. 2. GLC-MS analysis of the samples confirmed the presence of 3-methylglutaconate at >2 mol/mol creatinine with a similar *cis-trans* ratio.

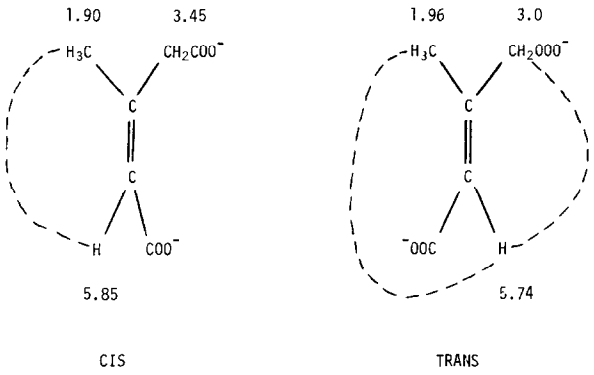

Fig.4. *Cis* and *trans* isomers of 3-methylglutaconate and their ^{1}H-NMR assignments at pH 5.7. Chemical shifts are given in ppm. The dotted lines show the couplings detected by the 3-D chemical shift correlated spectrum.

4. DISCUSSION

The 1-D NMR spectra of these siblings are very similar both qualitatively and quantitatively and, by comparison with urine spectra from both healthy individuals and patients with other metabolic disorders, appear to be diagnostic for HMG-CoA lyase deficiency [7,8,10].

The major abnormal metabolites, with the exception of 3-methylglutaconate, could be identified by comparison with spectra of authentic standards. However, a 3-methylglutaconate standard was not available. Six major resonances, all of which were singlets were unaccounted for in the spectra. Assignment of these peaks was significantly aided by the use of 2-D techniques. Identification of components whose NMR spectra consist solely of one or more (apparent) singlets is often extremely difficult in a multi-component system. The presence of multiplets simplifies identification of the type of group (e.g. CH_2 or CH_3) and double resonance techniques can be used to determine which multiplets originate from the same molecule. Such techniques are inappropriate for singlets or where coupling constants are very small (<2 Hz). However, long-range couplings (<2 Hz) may occasionally be detected in a 2-D spectrum particularly from unsaturated compounds. In the case of 3-methylglutaconate several long-range (4-bond) couplings could be detected in the COSY spectrum not readily apparent in the 1-D spectra. The 2-D spectrum also provides useful confirmation of the assignments made from the 1-D spectra since it reveals 4-bond couplings associated with 3-hydroxy-3-methylglutarate and 3-hydroxyisovalerate.

A preliminary report of this work has been presented [10].

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