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ORIGINAL ARTICLE

An NMR study of inclusion complexes formed by α-cyclodextrin and (*R*)- or (*S*)-α-lipoic acid

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Abstract A ^{1}H NMR study that explored the ability of α-cyclodextrin (α-CD) to preferentially bind (*R*)-α-lipoic acid is presented. The interaction between α-CD and (*R*)-α-lipoic acid was found to be stronger than that between α-CD and (*S*)-α-lipoic acid. Structures for the (*R*)-α-lipoic acid/α-CD and (*S*)-α-lipoic acid/α-CD inclusion complexes were constructed using restraints derived from ROESY spectra and MM2 molecular mechanics calculations. The models built for both complexes have the 1,2-dithiolane ring and the carboxyl moiety of α-lipoic acid oriented toward the secondary and primary hydroxy sides of α-CD, respectively.

Keywords Cyclodextrin · Inclusion complex · Lipoic acid · NMR · ROESY

Introduction

(*R*)-α-Lipoic acid (Fig. 1a), a compound found naturally in low amounts in food such as liver, spinach, and tomatoes, can effectively prevent oxidative stress in vivo [1–3]. Its 1,2-dithiolane ring is responsible for the antioxidant activity. Light, heat and alkaline conditions inactivate it. Additionally, it is poorly soluble in water [4]. Cyclodextrins (CDs) are expected to increase the solubility and stability of (*R*)-α-lipoic acid by forming (*R*)-α-lipoic acid/CD inclusion complexes, suggesting that such complexes can be used as nutritional (*R*)-α-lipoic acid supplements. CDs are cyclic oligosaccharides, containing six (α-CD, Fig. 1c), seven (*β*-CD), or eight (*γ*-CD) D-glucopyranose units [5, 6]. In aqueous solution, CDs can accommodate a variety of organic compounds in their central cavities and are therefore widely used to protect substrates from physical or chemical damage. Certain properties of the (*R*)-α-lipoic acid/*β*-CD inclusion complex have been determined, but its structure has not been characterized, because its rotating frame nuclear Overhauser effect spectroscopy (ROESY) spectrum contained only a few NOEs as (*R*)-α-lipoic acid was mobile in the *β*-CD cavity [7, 8]. α-CD has a smaller cavity than does *β*-CD, which suggests that α-CD should be able to maintain (*R*)-α-lipoic acid in a fixed orientation for structural determination. Although as noted above, (*R*)-α-lipoic acid/CD inclusion complexes have been reported, inclusion complexes with the non-natural enantiomer, (*S*)-α-lipoic acid (Fig. 1b), have not been studied. Herein, we report an NMR study of the α-CD inclusion complexes for (*R*)- and (*S*)-α-lipoic acid, and show that α-CD preferentially binds (*R*)-α-lipoic acid. Additionally, using restraints derived from the corresponding ROESY spectra, we built energy-minimized structures for the α-CD complexes with (*R*)- or (*S*)-α-lipoic acid.

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Materials and methods

Materials

α-Cyclodextrin (CAVAMAX W6 Food) and (*R*)- and (*S*)-α-lipoic acid were supplied by Wacker chemical Co. and Toyo hakko Co., Ltd., respectively. Deuterium oxide, with an isotopic purity of 99.95%, was purchased from Merck Co.

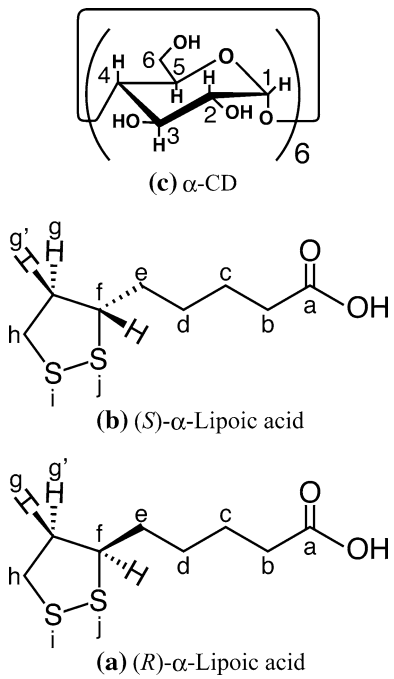

Fig. 1 Structures of **a** (*R*)-α-lipoic acid, **b** (*S*)-α-lipoic acid, and **c** α-cyclodextrin

Sample preparation for NMR studies

Mixtuers of (*R*)- or (*S*)-α-lipoic acid (2 mM) and α-CD (2 mM) were sonicated in D_2O (1 mL) for 20 min and insoluble materials were removed by filtration.

^{1}H-NMR spectroscopy

One- and two-dimensional ^{1}H-NMR spectra were recorded at 25 °C using Bruker Avance 600 and Varian VXR-500S spectrometers operating at 600.13 and 499.843 MHz, respectively. All spectra were recorded using the manufacturer's suggested pulse sequences and procedures. The ^{1}H chemical shift of HDO ($\delta = 4.70$ ppm) served as the internal standard. The rotating frame nuclear Overhauser effect spectroscopy (ROESY) spectra were obtained with a mixing time of 300 ms and 32 scans for each t1 increment (256 in total). HDO was suppressed by selective irradiation during the repetition delays.

The estimated structures of the α-lipoic acid/α-CD complexes

To elucidate a plausible structure for the α-lipoic acid/α-CD complexes, molecular mechanics calculations were performed using ChemBio3D Ultra 12.0.3 (CambridgeSoft Corporation, 2010) software with a modified Allinger's MM2 force field. Each α-lipoic acid enantiomer was initially placed into the α-CD cavity with its 1,2-dithiolane ring and carboxyl moiety oriented toward the secondary and primary hydroxy faces of α-CD, respectively, and then the energy of each structure was minimized. In addition, several other initial (*R*)- and (*S*)-α-lipoic acid positions were subjected to energy minimization, and, for all runs, the final structures were nearly the same.

Results and discussion

Figure 2 shows the α-lipoic acid region of the ^{1}H NMR spectra of (*R*)-α-lipoic acid alone, (*R*)-α-lipoic acid in the presence of α-CD, and (*S*)-α-lipoic acid in the presence of α-CD in D_2O. The H-b and H–d resonances in the spectrum of (*R*)-α-lipoic acid in the presence of α-CD are shifted upfield and downfield, respectively, compared with their positions in the spectrum of (*R*)-α-lipoic acid alone, suggesting that H-b and H-d interact with different parts of α-CD, i.e., they are oriented on opposing sides of α-CD. Additionally, in the presence of α-CD, the ^{1}H resonances of

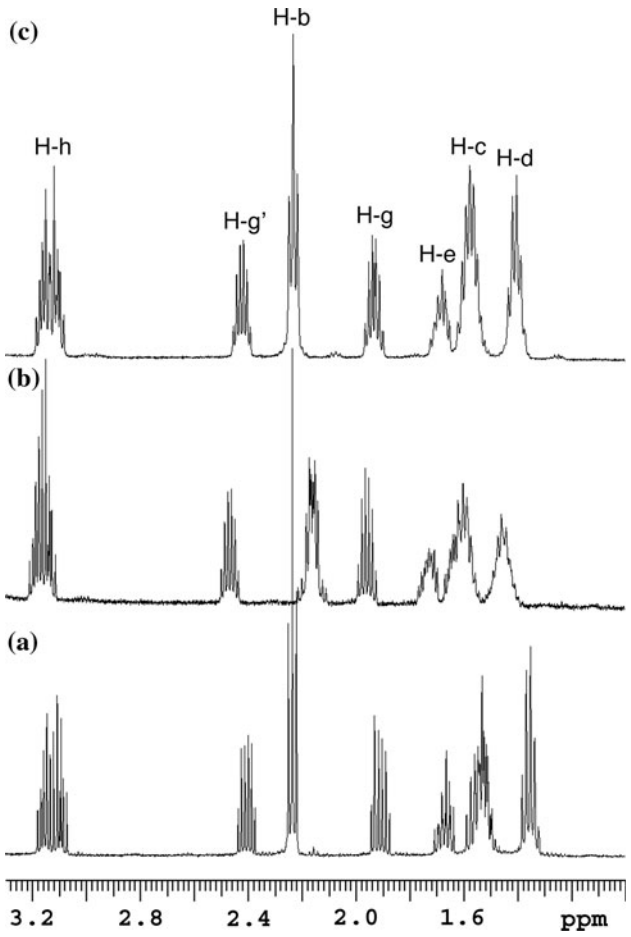

Fig. 2 α-Lipoic acid regions in the ^{1}H NMR spectra of **a** (*R*)-α-lipoic acid alone, **b** (*R*)-α-lipoic acid in the presence of α-CD, and **c** (*S*)-α-lipoic acid in the presence of α-CD in D_2O at 25 °C

(*R*)-α-lipoic acid are shifted to a greater extent than are those of (*S*)-α-lipoic acid, suggesting that the overall interaction between α-CD and (*R*)-α-lipoic acid is stronger than that between α-CD and (*S*)-α-lipoic acid. Notably, the resonances for H-b of (*R*)-α-lipoic acid in the presence of α-CD show a complicated splitting pattern, whereas those of (*S*)-α-lipoic acid in the presence of α-CD and (*R*)-α-lipoic acid alone present as simple triplets (Fig. 3). Additionally, splitting patterns of the other resonances for both enantiomers in the presence of α-CD are nearly identical. Because the H-b resonances are magnetically non-equivalent in the (*R*)-α-lipoic acid/α-CD spectrum, a rotational barrier around a C–C bond may exist, possibly caused by a hydrogen bond between the carboxyl group of (*R*)-α-lipoic acid and a hydroxy group of α-CD. The stereochemical effect of the chiral C-f may cause the differences in the position of the carboxyl group for enantiomers even though the distance between the C-f and C-b carbons is far apart.

If each of the α-lipoic acid enantiomers is bound within the α-CD cavity, NOE correlations between their protons and those of α-CD (H-3, H-5, or H-6) would be observed, and it would then be possible to orient each α-lipoic acid in the α-CD cavity using the restraints derived from the assigned NOE intensities [9, 10]. NOE correlations between protons of each enantiomer and protons of α-CD were observed in the corresponding ROESY spectrum. Figures 4 shows the assigned ROESY spectrum with the NOE correlations that connect protons of (*R*)-α-lipoic acid with protons of α-CD diagrammed. The intensity of the NOE correlation between the H–d of (*R*)-α-lipoic acid and the H-3 of α-CD is larger than that between the H–d of (*R*)-α-lipoic acid and the H-5 of α-CD. The intensity of the NOE correlation between the H-b of (*R*)-α-lipoic acid and the H-5 of α-CD is larger than that between the H-b of (*R*)-α-lipoic acid and the H-3 of α-CD. The intensity of the NOE correlation between the H-c of (*R*)-α-lipoic acid and the H-3 of α-CD is similar to that between the H-c of (*R*)-α-lipoic acid and the H-5 of α-CD. These results strongly suggest that the 1,2-dithiolane ring and the carboxyl moiety of (*R*)-α-lipoic acid are oriented toward the secondary and

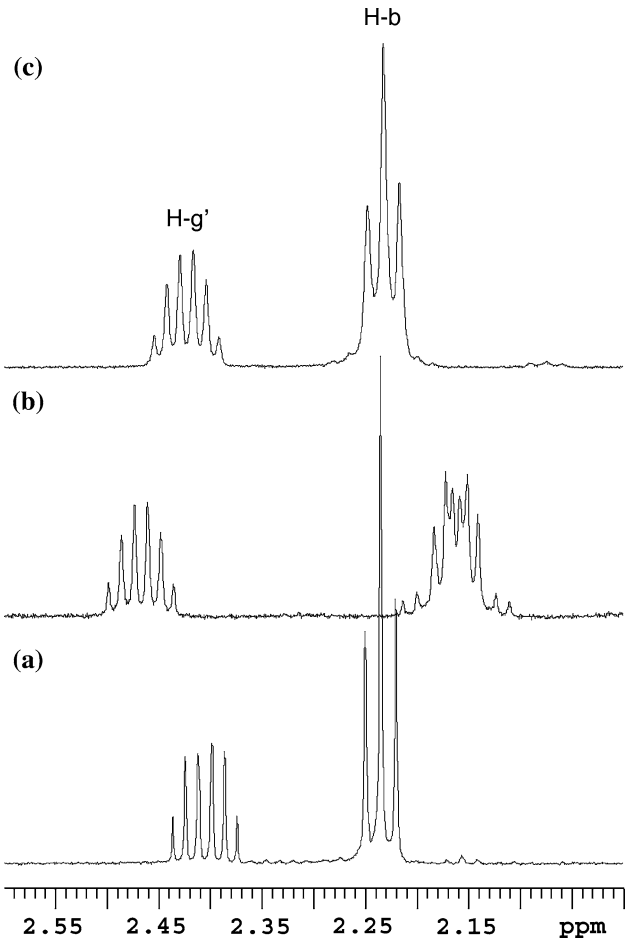

Fig. 3 H-g′ and H-b resonances in the ^{1}H NMR spectra of **a** (*R*)-α-lipoic acid alone, **b** (*R*)-α-lipoic acid in the presence of α-CD, and **c** (*S*)-α-lipoic acid in the presence of α-CD in D_2O at 25 °C

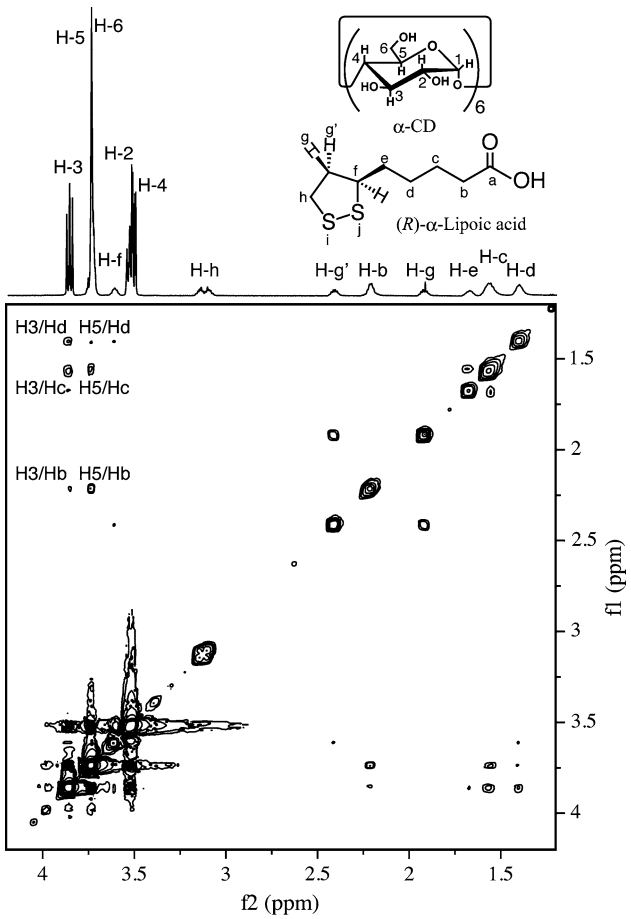

Fig. 4 ROESY spectrum of the (*R*)-α-lipoic acid/α-CD complex

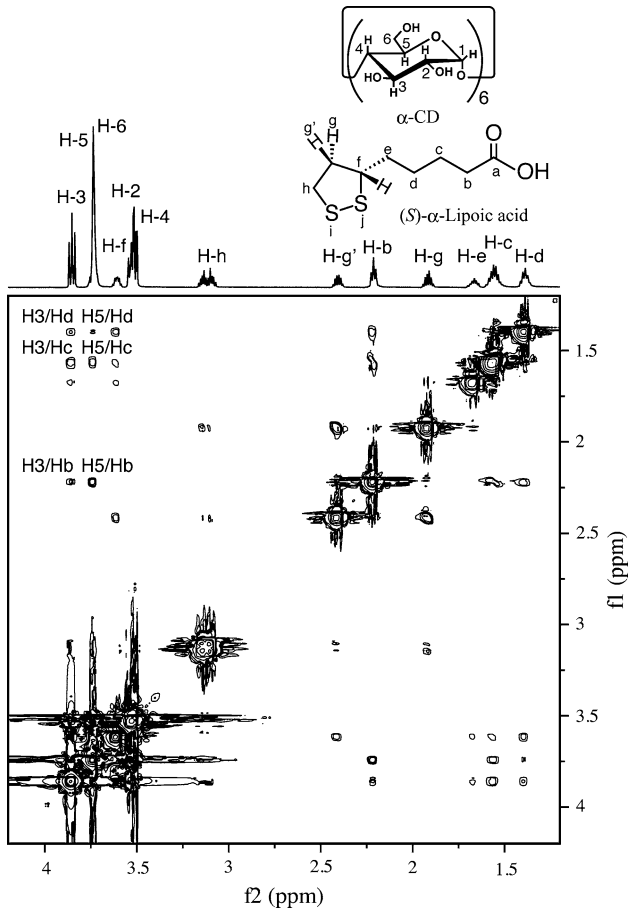

Fig. 5 ROESY spectrum of the (*S*)-α-lipoic acid/α-CD complex

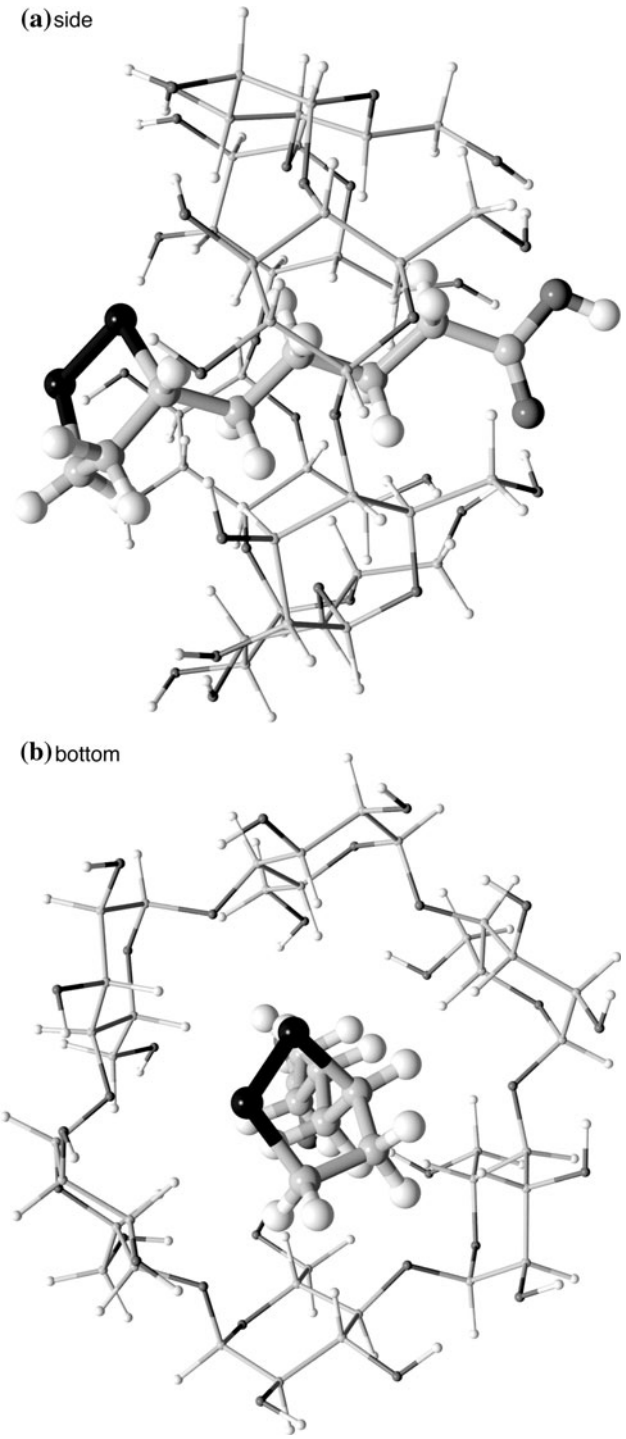

Fig. 6 Estimated structure of the (*R*)-α-lipoic acid/α-CD complex; **a** side view, **b** view from the secondary hydroxy side

primary hydroxy sides of α-CD, respectively. A ROESY spectrum of (*S*)-α-lipoic acid in the presence of α-CD was also acquired (Fig. 5). The pattern of NOE correlations in the (*S*)-α-lipoic acid/α-CD spectrum is similar to that in the (*R*)-α-lipoic acid/α-CD spectrum but the NOE intensities for former are weaker than those for the latter. In general, NOE intensities depend on the distance between the interacting groups, which for a noncovalent complex such as a CD/guest inclusion complex, is partially dictated by the binding strength of the complex. Therefore, the stronger NOE intensities found in (*R*)-α-lipoic acid/α-CD spectrum suggest, as did the larger CD-induced chemical shift changes, that the (*R*)-α-lipoic acid/α-CD interaction is stronger than the (*S*)-α-lipoic acid/α-CD interaction.

Structure for the two α-lipoic acid/α-CD inclusion complexes based on the NOE constrains obtained from their ROESY spectra were built using the MM2 molecular mechanics module in ChemBio3D (Figs. 6, 7). For both complexes, the 1,2-dithiolane rings are approximately perpendicular to the C6 axis of α-CD, which explains why no NOE correlations were found for the protons of the 1,2-dithiolane ring and the protons of α-CD. The total steric energies for the (*R*)- and (*S*)-α-lipoic acid inclusion complexes are 67.0 and 68.6 kcal/mol, respectively, indicating that the former is the more stable complex in vacuo. The values for the energies agree with the NMR data that suggested that (*R*)-α-lipoic acid bound somewhat more tightly to α-CD.

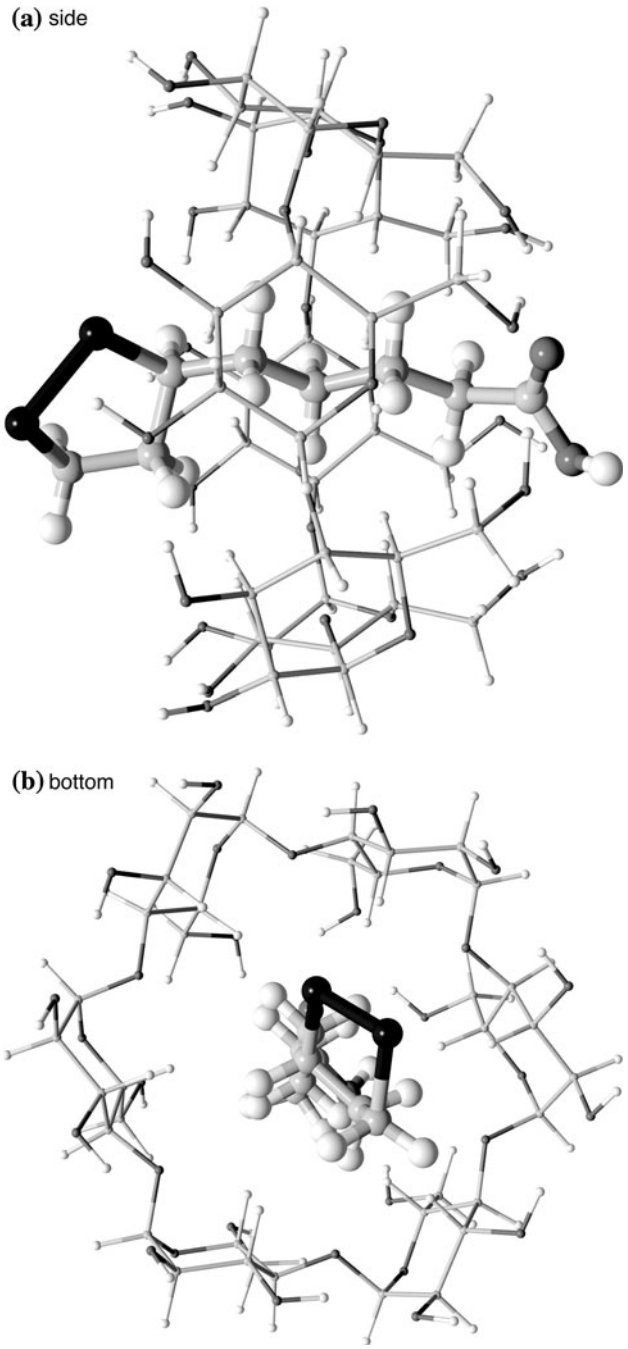

Fig. 7 Estimated structure of the (*S*)-α-lipoic acid/α-CD complex; **a** side view, **b** view from the secondary hydroxy side

Conclusions

The interaction of α-CD with the naturally occurring (*R*)-α-lipoic acid is stronger than that with the non-naturally occurring enantiomer, (*S*)-α-lipoic acid. Structures for the two α-lipoic acid/α-CD inclusion complexes were built using NOE-derived restraints and MM2 molecular mechanics calculations. In both structures, the 1,2-dithiolane ring and the carboxyl moiety of the α-lipoic acid are oriented toward the secondary and primary hydroxy sides of α-CD, respectively.

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