under practical, experimental conditions with nonideal pulses. With this modification the TOCSY experiment carries the promise to facilitate ^{1}H spin system identifications in macromolecules and to enable such studies with bigger molecules than would be possible with presently available techniques.

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Hg_{18}–Metallothionein

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We report the formation of a novel mercury–protein complex, namely Hg_{18}–metallothionein, from rabbit liver metallothionein (MT) isoform 2. This new species is characterized by a strong circular dichroism (CD) intensity under the thiolate-to-mercury charge-transfer bands in the 240–360 nm region. The presence of this unusually intense CD spectrum suggests that Hg_{18}-MT 2 adopts a specific 3-dimensional structure not found previously for MT species, rather than the random coil structure expected for such a high metal loading.

Metallothioneins (MT) are low molecular weight, cysteine-rich proteins containing 20 SH groups per molecule.[1] MT binds a wide range of metal ions both in vivo and in vitro.[2,3] The stoichiometric ratio for the sum of Zn and Cd binding to MT is 7,[3] while for Cu[3-5] and Ag[3] the ratio is 12. Few spectroscopic, structural and stoichiometric data are available for Hg,[3,6-9] despite the importance of this element in metal toxicity. The only species reported to date for Hg is Hg_7-MT.[3,6,7]

CD spectral intensity with group 11 and 12 metals bound to metallothionein arises from ligand-to-metal charge transfer (LMCT).[4,6,10] CD spectra probe the chirality of the whole metal binding site cage. The absence of aromatic amino acids results in a spectral window in the wavelength region of these charge-transfer transitions.[4,10]

Figure 1 shows that isodichroic formation of Hg_7-MT[11] takes place when Hg is added to apo-MT 2 at pH 2.4, resulting in band maxima at 310 nm (+) and 270 nm (−), and the presence of a symmetrical well in intensity between 260 and 290 nm in the contour level diagram. The magnetic circular dichroism (MCD) spectrum suggests that Hg_7-MT formed at pH 2.4[14] does not adopt a geometry similar to that of Cd_7-MT.[15,16]

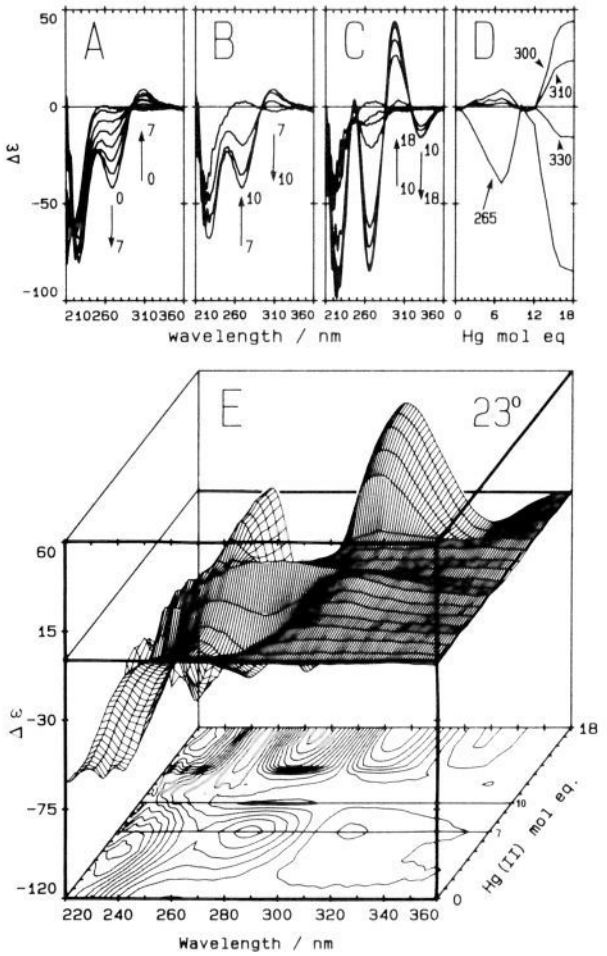

Figure 1. CD spectra recorded during a titration of rabbit liver apo-MT 2 with Hg(II) at pH 2.4. (A) 0–7, (B) 7–10, (C) 10–18 mol equiv of Hg; (D) Intensities as a function of mol equiv of Hg; (E) 0–18 mol equiv, the third axis in (E) is mol equiv of Hg.

As more Hg is added (up to 10 mol equiv), the Hg_7-MT CD signal diminishes as a new, but weaker, signal forms isodichroically (Figure 1B). Surprisingly, once 12 mol equiv of Hg(II) have been added, a very strong CD spectrum begins to form isodichroically (Figure 1C), reaching a maximum intensity at 18 mol equiv. Figure 1D shows changes in intensity as a function of mol equiv of Hg added at the band maxima for Hg_{18}-MT 2 (300 (+), 330 (−), 265 nm (−)) and also at 310 nm (band maximum (+) for Hg_7-MT). No further changes are found with up to 40 mol equiv of Hg. The complex is stable between pH 2 and 6.9; the CD spectral envelope collapses above pH 7.

A new Hg_7-MT species forms at 2 °C and pH 2.4 (Figure 2) unlike Cd_7-MT which does not form below 5 °C.[17] However, significantly, only a small fraction of the Hg_{18}-MT expected forms as more Hg is added. Subsequent warming to 37 °C for 10 min results in complete formation of Hg_{18}-MT (Figure 2C). The

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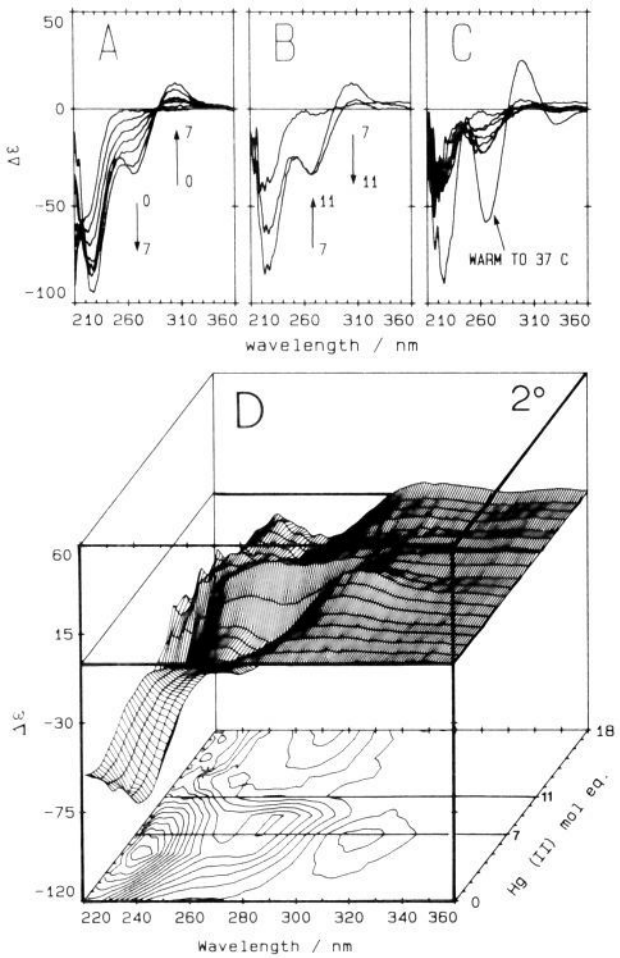

Figure 2. CD spectra recorded at 2 °C during a titration of rabbit liver apo-MT 2 with Hg(II) at pH 2.4. (A) 0–7 (0, 1, 2, 3, 4, 5, 7); (B) 7–11 (7, 9, 11); (C) 11–18 (11, 12, 13, 14, 15, 17, 18) mol equiv of Hg at 2 °C, and the spectrum for 18 mol equiv measured at 37 °C; (D) 0–18 mol equiv, the third axis in (D) is mol equiv of Hg.

reaction is not reversible, and the Hg_{18}-MT CD spectrum remains if the temperature is subsequently reduced to 2 °C. There is no free Hg at either temperature.

The spectrum observed for Hg_{18}-MT 2 in this wavelength region arises from $RS^- \rightarrow Hg^{2+}$ transitions. The high CD intensity observed for Hg_{18}-MT must arise from a strongly chiral environment for the Hg–S bonds. It is not possible for such an intensely dichroic signal to be generated if the structure of Hg_{18}-MT was a random coil. Because the CD spectrum is specifically sensitive (due to exciton coupling effects[10]) to formation of clustered species, like Cd_4-SR_{11},[4,10,17,18] a 3-dimensional structure is necessary in order to generate the CD spectrum observed when 18 Hg atoms are bound to the 20 thiolate groups in MT 2 below pH 7. This is highly unexpected. There is no evidence of dimer formation,[19] and it is unlikely that Hg_{18}-MT adopts the Cd_7S_{20}, 2-domain structure, because $Hg_{18}S_{20}$ cannot form the necessary Hg–S–Hg bridges. We suggest that the CD signal arises from stacking of the Hg–S bonds in the protein either in a hairpin-like structure or in one that involves extensive coiling of the peptide chain. In the absence of aromatic amino acids, we only find exciton bands related to the Hg–S chromophore. Reports for both Cd_7-MT[10] and Co_7-MT[20] with EPR, CD, and MCD techniques support the view that metal-related transitions are reliable markers for the presence of well-defined, metal–thiolate structures.

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Enantioselective Synthesis of Dihydropyridine Sulfones[1]

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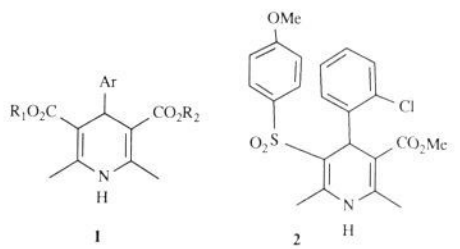
Calcium channel antagonistic 4-aryl-1,4-dihydropyridine-3,5-carboxylic acid diesters[2] (**1**) are important cardiovascular drugs



1 **2**

which inhibit smooth and cardiac muscle contractions by blocking the influx of calcium ions through plasma membrane channels.[3] When the two ester groups are different, C_4 of the dihydropyridine ring becomes chiral, and stereoselectivity of antagonism is observed.[4] Due to the many additional pitfalls which exist when assessing the pharmacological properties of racemic drugs[5] and the changing perception of this problem on the part of regulatory agencies, the preparation and biological evaluation of single isomers have become mandatory.

In the area of dihydropyridines (DHP), optically active isomers have been produced either by classical resolution of a monoacid or by separation of diastereomeric esters.[7] A potentially useful asymmetric synthesis of DHP derivatives involving enantioselective addition of organometallic reagents to the 4-position of a pyridine has been demonstrated in a simple case.[8]

Since we had identified the racemic sulfonyl DHP, **2**, as a potent, orally bioavailable and long acting antihypertensive agent in animal models, we decided to synthesize the corresponding

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