CO derivatives are similar to that of cyt *c*-CO at pH 13.7 (414 nm, 2.54×10^5 M^{-1} cm^{-1}).[33] In contrast, cytochrome P-450-CO, with thiolate-Fe(II)-CO ligation, has an absorption maximum at 446 nm.[34] These results indicate that residue 80, not His18, is displaced from Fe(II) upon reaction with CO.

We have shown that replacement of the axial methionine in cytochrome *c* can lead to dramatic changes in the heme reduction potential; that cytochrome *c* refolding can be achieved in the absence of a position-80 ligand; and that the ligand-binding properties of cytochrome *c* can be significantly altered. The position-80 cytochrome *c* derivatives should be useful in studies of long-range donor-acceptor electronic couplings in proteins, and work in this area is underway.

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Helix Formation of Melittin on Poly(L-glutamic acid) and Poly(D-glutamic acid)

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Melittin consists of 26 amino acid residues with six positive and no negative charges: H_3N^+Gly-Ile-Gly-Ala-Val-Leu-Lys^+-Val-Leu-Thr_{10}-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile_{20}-Lys^+-Arg^+-Lys^+-Arg^+-Gln-Gln-NH_2.[1] Many investigators have studied conformational changes of melittin induced by high pH,[2-4] high salt,[4,5] and high melittin concentration,[2,4-6] as well as by the addition of surfactants,[3,7] lipid/membrane,[8] methanol,[9] and calmodulin.[10] However, we now report unprecedented conformational changes of melittin lying on anionic polymers.

Melittin (Sigma) was decolored by gel permeation chromatography with 10% acetic acid as the eluent.[3] The protein concentration was determined with $\epsilon_{280} = 5600$.[2,3] Poly(L-glutamic acid) (PLGA) and poly(D-glutamic acid) (PDGA) were used as the anionic polymers (both from Sigma). The circular dichroism (CD) spectra of melittin-PLGA (A) and melittin-PDGA (B) systems, measured on a JASCO J-600 instrument, are shown in Figure 1. When we subtracted the spectrum of polymer from that of the melittin-polymer mixture, we obtained the spectrum of melittin alone, which was indicative of α-helical structure in both cases. If such a spectrum of melittin was observed only in the melittin-PLGA mixture, there remained a possibility that some parts of the polymer also adopted helical structures induced by the protein. However, the spectrum of melittin alone with the same intensity was observed also in the melittin-PDGA mixture (if the helical structure of PDGA is induced by melittin, the negative intensity of the spectrum should decrease more or less). Therefore, the present results clearly indicate that the helices of melittin are formed on these polymers irrespectively of their optical activity.

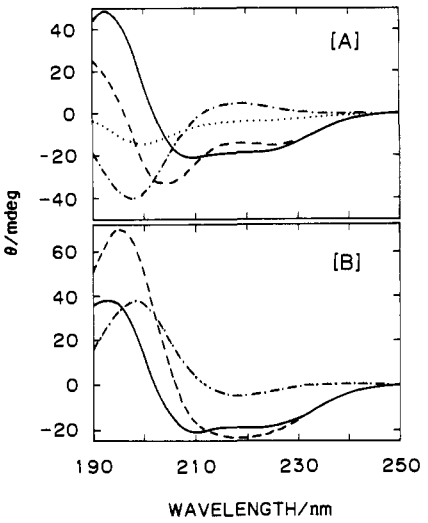

Figure 1. CD spectra of melittin-PLGA (A) and melittin-PDGA (B) systems at neutral pH and 25 °C. Each shows CD spectra of a melittin-polymer mixture (---), polymer along (-·-), and the difference spectrum (—) between them which corresponds to melittin alone in the presence of PLGA (A) and PDGA (B). The dotted curve in part A indicates the spectrum of melittin in the absence of PLGA. Polymerization degree of polymer: 88 (PLGA) and 90 (PDGA). Polymer concentration: 0.52 mM (residue). Melittin concentration: 20 μM (0.52 mM in residue concentration). Light path of cell: 2 mm.

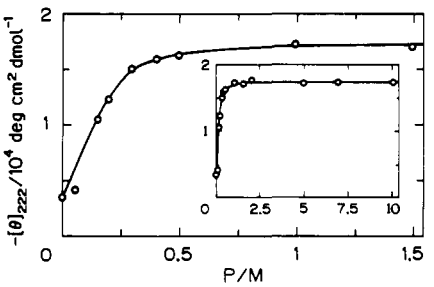

Figure 2. The dependence of $[\theta]_{222}$ for melittin alone on P/M (see text) in melittin-PLGA (polymerization degree: 88) system. The inset shows the data over a wide range of P/M.

The changes of CD spectra of melittin depended on the mixing ratio, P/M (P, PLGA or PDGA; M, melittin, both in residue concentration). Figure 2 shows the dependence of $[\theta]_{222}$ for melittin alone on P/M. The value of $[\theta]_{222}$ abruptly increased until $P/M = 0.4$ (the solution was slightly turbid below $P/M = 0.3$), attaining an approximately constant magnitude of $-18\,000$ to $-17\,000$ deg cm^2 dmol^{-1} at P/M higher than 1. The saturated residue ellipticity is appreciably larger than that at pH 12 and is smaller than that in sodium dodecyl sulfate.[3] We used PLGA with polymerization degrees of 88 and 380 and PDGA with polymerization degrees of 90 and 380. However, the dependence

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of $[\theta]_{222}$ on P/M was not influenced by the differences in the polymerization degree and in the optical activity of the polymers. When we chose $[\theta]_{222} = -2000$ and -28400 deg cm^2 dmol^{-1} for the disordered and helical structures of melittin, respectively,[3,11] the proportion of helical structure was estimated to be 75% beyond $P/M = 1$.

As is known, PLGA and PDGA adopt helical structures at acidic pH. However, these polymers did not induce helix formation in melittin at pH 2.3 because of loss of their original negative charges (the solution was clear). On the contrary, upon subtracting the spectrum of PLGA or PDGA from that of the mixture, we obtained the spectrum of melittin alone, indicative of a more disordered structure (−26 000 deg cm^2 dmol^{-1} around 195 nm) at the acidic pH than at neutral pH (Figure 1A). This might suggest that melittin has a slight amount of helices at neutral pH.

On the other hand, helix formation was not induced in melittin by the coexistence (<5 mM) of a simple anionic amino acid, glutamic acid. The polymers appear likely also to provide a place for the helix formation of melittin. A similar situation can be anticipated for melittin in surfactant solutions. The nonpolar tails of surfactants bound to cationic residues of melittin must strongly interact with one another, forming a micelle-like structure.[3] This micelle-like aggregate appears likely to supply a similar place for the helix formation. At neutral pH, some of six cationic charges of melittin might be neutralized on PLGA and PDGA. Then, the helical moiety, with four continuous cationic residues in the C-terminal, are considered to be entwined with PLGA or PDGA. Probably related to this, the disordered state might be required for the polymers to twine around the helical rod. Interestingly, the value of $[\theta]_{222}$ becomes almost constant above $P/M = 1$ (Figure 2), while polymerization degrees of the present polymers are much larger than the residue number, 26, of the protein. We speculate that a considerable amount of helical melittin molecules hang on each of the homopolypeptide chains.

The present results tend to indicate that the short polypeptide melittin presents a useful model for the studies of polypeptide–polypeptide interactions as well.

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Catalytic Asymmetric Aldol Reactions. Use of a Chiral (Acyloxy)borane Complex as a Versatile Lewis Acid Catalyst

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The development of chiral catalysts that mediate the asymmetric aldol condensations in a highly stereocontrolled and truly catalytic manner has been a challenging goal in synthetic organic chemistry. Although much fascinating chemistry has been exploited on this problem, which provided excellent methods for chirality transfer from chiral substrates or auxiliaries to prochiral molecules, it has not led to an ultimate means of propagating chirality with a nonstoichiometric amount of a chiral source, except in a few special cases.[1] We report now a successful solution to this problem.

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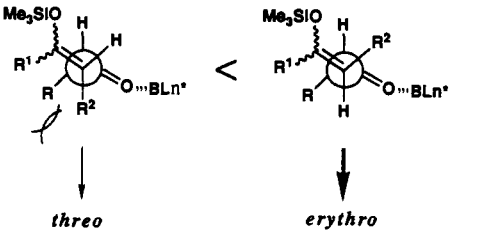
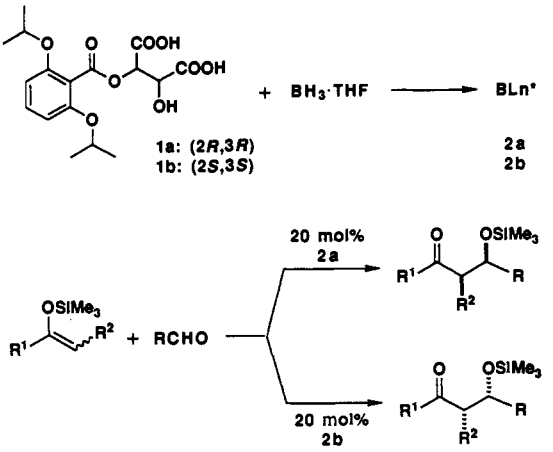


Figure 1. Extended transition state model.

Scheme I



Our method uses a chiral (acyloxy)borane (CAB) complex[2] as a Lewis acid catalyst for the Mukaiyama condensation of simple chiral enol silyl ethers of ketones with various aldehydes.[3] This CAB-catalyzed aldol process allows the formation of adducts in a highly diastereo- and enantioselective manner (up to 96% ee) under mild reaction conditions. Furthermore, the reactions are catalytic, thus only 20 mol % of catalyst is needed for efficient conversions, and the chiral source is recoverable and reusable.

Chiral (acyloxy)borane complex **2** was easily prepared in situ from tartaric acid derivative **1** and BH_3·THF complex in propionitrile solution at 0 °C[4] (Scheme I). The aldol reactions of ketone enol silyl ethers with aldehydes were promoted by 20 mol % of this catalyst solution at low temperature.[5] After a usual workup, the crude product mixture (mostly silylated β-hydroxy ketones) was treated with diluted hydrochloric acid to afford desilylated aldol adducts. Product diastereomer ratios were determined by analytical HPLC and ^{1}H NMR spectroscopy of the adducts and/or the corresponding MTPA esters. The stereochemical assignments (relative stereochemistries) were made from the analyses of the ^{1}H NMR spectra, and the absolute configurations were determined by comparison of the specific rotation values with those of the literature. Some results are summarized in Table I.

The relative stereochemistry of the major adducts was assigned as erythro, and predominant *re*-face attack of enol ethers at the aldehyde carbonyl carbon was confirmed in cases where a natural tartaric acid derivative was used as a Lewis acid ligand. The use of an unnatural form of tartaric acid as a chiral source afforded the other enantiomer as expected (entry 8). Almost perfect asymmetric inductions were achieved in the erythro adducts, reaching 96% ee, although a slight reduction in both the enantio-

(2) For precedent applications of CAB catalysts to asymmetric reactions, see: (a) Furuta, K.; Miwa, Y.; Iwanaga, K.; Yamamoto, H. *J. Am. Chem. Soc.* **1988**, *110*, 6254. (b) Furuta, K.; Shimizu, S.; Miwa, Y.; Yamamoto, H. *J. Org. Chem.* **1989**, *54*, 1481. (c) Furuta, K.; Kanematsu, A.; Yamamoto, H.; Takaoka, S. *Tetrahedron Lett.* **1989**, *30*, 7231.

(3) For a review of the Mukaiyama aldol reaction, see: Mukaiyama, T. *Org. React. (N.Y.)* **1982**, *28*, 203.

(4) Tartaric acid **1** was prepared by the monoacylation of dibenzyl tartrate followed by hydrogenolysis.

(5) The use of 10 mol % catalyst for the reaction resulted in a significant decrease in reactivity.

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