# Theoretical Analysis and Prediction of Catalysts for Oxidative Decarboxylation of Melanin-Concentrating Hormone

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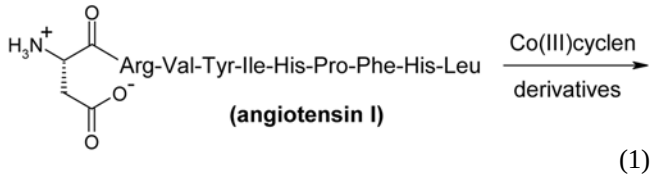
In a previous study, a catalyst (**A**) was discovered for oxidative decarboxylation of melanin-concentrating hormone (MCH). To explain the catalytic action and to predict the structure of a new catalyst with improved activity, docking simulations were carried out for the complex formed between **A** and MCH. The simulations suggested that the three terminal groups of **A** form a hydrophobic pocket and that van der Waals interactions between the hydrophobic pocket and MCH play a role in stabilizing the MCH-**A** complex. Consequently, a new catalyst (**B**) was designed and synthesized in expectation of improved catalytic activity resulting from enhanced van der Waals interactions. The new catalyst, however, showed slightly lower catalytic activity. Lack of the accurate solution structure of MCH may be one of the factors associated with difficulties in prediction of improvement in catalytic activity by purely theoretical means. The results, however, revealed that variation of the acyl portion of the hydroxyproline portion may lead to improved catalysts.

**Key Words :** Melanin-concentrating hormone, Oxidative decarboxylation, Co(III) complex of cyclen

## Introduction

We have reported the first catalysts for oxidative decarboxylation of an *N*-terminal Asp residue of oligopeptides by using human angiotensin I, a decapeptide, and angiotensin II, an octapeptide, as substrates.[1] Thus, the *N*-terminal Asp of angiotensin I or angiotensin II was converted to pyruvate (Pyr) residue by the synthetic catalysts as indicated by the reaction of eq 1. Here, the Co(III) complex of cyclen (Co(III)cyclen) was used as the catalytic center for the oxidative decarboxylation of the *N*-terminal Asp and binding sites that recognize angiotensin I or angiotensin II were selected from chemical libraries.

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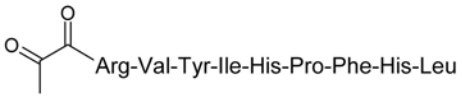
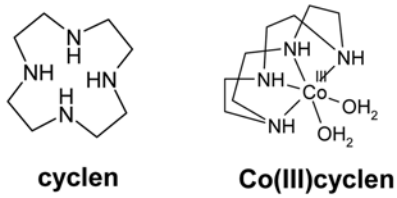
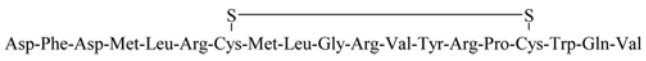



Catalysts for oxidative decarboxylation of an *N*-terminal Asp residue were unprecedented in both biotic and abiotic systems. In addition, catalysts for destruction of disease-related oligopeptides may be used as new drugs. Therapeutic manipulation of the pathway involving angiotensin I and angiotensin II has become very important in treating hypertension and heart failure.[2-4] In this regard, we attempted to discover peptide-cleaving catalysts selective for other disease-related peptide hormones containing *N*-terminal Asp.

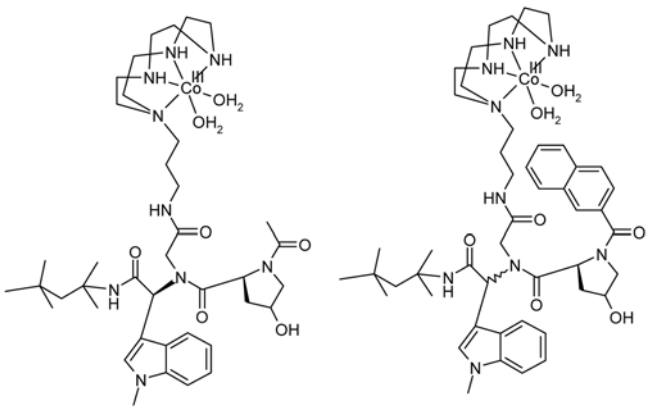
As the next target for discovery of the target-selective oligopeptide-cleaving catalysts, we chose human melanin-concentrating hormone (MCH). MCH is a cyclic oligopeptide containing 19 amino acid residues with the *N*-terminal residue being Asp. MCH, a neuropeptide highly expressed in the lateral hypothalamus, has an important role in the regulation of energy balance and body weight in rodents and is a target of designing drugs for obesity.[5-8]




**MCH**

From chemical libraries containing about 19000 derivatives of Co(III)cyclen, only **A** was found to have catalytic activity for oxidative decarboxylation of the *N*-terminal Asp of MCH.[9]

In view of the potential utility of the catalyst for oxidative decarboxylation of MCH as catalytic drugs for obesity, it is desirable to provide theoretical explanation for the activity of **A** and to discover other compounds possessing improved catalytic activity. For these purposes, we have carried out docking simulation for the complex formed between **A** and MCH and have synthesized a derivative of **A** on the basis of the results of the docking simulations. In the present study, results of the docking simulations and synthesis and activity measurement of the new catalyst (**B**) are described.

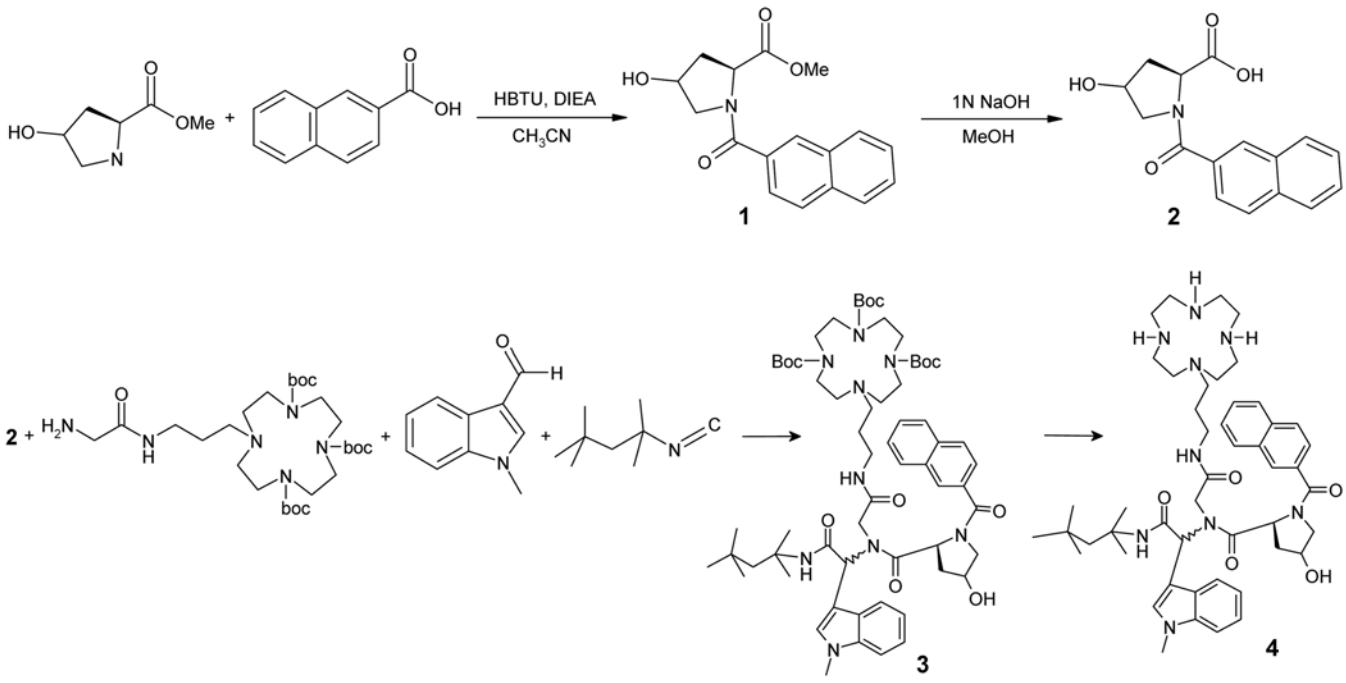


**A** **B**

## Experimental Section

**Computational Methods.** The geometry of **A** was fully optimized with density functional calculations at B3LYP/3-21G* level of theory using JAGUAR 4.1 program.[10] The optimized structure was then docked onto the surface of the *N*-terminus of MCH. This docking simulation was carried out with AutoDock 3.0.5 program,[11] which combines a rapid energy evaluation through precalculated grids of affinity potentials with the Lamarckian genetic algorithm to find suitable binding positions for a ligand on a protein receptor. Although the protein structure has to be fixed, the program allows torsional flexibility of a ligand. Three dimensional structure of MCH has not been reported so far although its conformational features were addressed with NMR and computational methods.[12] Therefore, the starting coordinates of MCH were prepared from comparative protein structure modeling using the Omega-conotoxin TxVII (PDB ID: 1F3K) as a structural template. To find this remote homolog of MCH, we used the fold recognition by 123D threading algorithm[13] because no sequence homolog was found in PDB. The threading method attempts to place the target sequence onto the template backbone, followed by the evaluation of the newly generated structure with mean-force potentials or environmental classifications. The amino acid sequences of MCH and the template were retrieved from the SWISS-PROT protein sequence data bank (http://us.expasy.org/sprot/; accession numbers P20382 and P56714, respectively). Sequence alignment between the target and the template was then derived with the CLUSTAL W package.[14] Based on this sequence alignment, 3D structures of MCH were constructed using the MODELLER 6v2 program.[15] In the docking simulation between MCH and **A**, we used AMBER force field parameters[16] for MCH. Due to the lack of potential parameters associated with Co(III) complex, RHF/6-31G* atomic partial charges were derived by applying the RESP methodology to the energy-minimized structure of **A**,[17] to be consistent with the standard AMBER force field. Of the conformations of **A** obtained from 100 docking runs on the surface of the *N*-terminus of MCH, clustered together were the results differing by less than 1.5 Å in positional root-mean-square deviation. The most stable MCH-**A** complex was then selected for further analysis.

**Preparation of B.** As summarized in Scheme 1, to the stirred solution of *trans*-4-hydroxy-*L*-proline methyl ester (0.46 g, 3.2 mmol) in $CH_3CN$ (50 mL) were added 2-naphthanoic acid (0.50 g, 2.9 mmol) and DIEA (1.5 mL, 8.7 mmol). To the reaction mixture was added HBTU (1.3 g, 3.6 mmol) and the mixture was stirred at 0 °C for 2 hours. The solution was evaporated and the residue was dissolved in EtOAc (50 mL). The EtOAc solution was washed with 5%




**Scheme 1**. Synthetic route to **4**.

aq. citric acid (25 mL), 5% aq. $Na_2CO_3$ (25 mL), and brine (30 mL), and then dried over $Na_2SO_4$. The solvent was evaporated off, and column chromatography afforded 4-hydroxy-1-(naphthalene-2-carbonyl)-pyrrolidine-2-carboxylic acid methyl ester (**1**) as a white solid. $R_f$ 0.3 (EtOAc only); $^1$H NMR (300 MHz, $CDCl_3$): 8.1 (s, 1H), 7.8 (d, 2H), 7.7 (m, 1H), 7.6 (m, 2H), 4.7 (br, 1H), 3.9 (d, 1H), 3.7 (s, 3H), 3.6 (d, 2H), 2.45 (m, 1H), 2.38 (m, 2H). To the solution of **1** (0.70 g, 2.3 mmmol) in MeOH (25 mL) was added aq. NaOH (1 N, 25 mL). The reaction mixture was stirred for 2 hours. The solvent was evaporated off, the residue was dissolved in 10% aq. citric acid, and pH was adjusted to 5. After the solution was extracted with EtOAc (50 mL × 3) and the organic layer was washed with brine (50 mL), dried over $Na_2SO_4$, and evaporated, 4-hydroxy-1-(naphthalene-2-carbonyl)-pyrrolidine-2-carboxylic acid (**2**) was obtained as a white solid. $^1$H NMR (300 MHz, $CDCl_3$): 8.1 (s, 1H), 7.8 (d, 2H), 7.7 (m, 1H), 7.6 (m, 2H), 4.7 (br, 1H), 3.9 (d, 1H), 3.6 (d, 2H), 2.45 (m, 1H), 2.38 (m, 2H). Compound **2** (0.17 g, 0.60 mmol), 10-[3-(2-amino-acetylamino)-propyl]-1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester (0.34 g, 0.60 mmol),[18] 1-methyl-1*H*-indole-3-carbaldehyde (0.14 g, 0.89 mmol) were dissolved in MeOH (10 mL). Ten minutes later, 2-isocyano-2,4,4-trimethyl-pentane (100 $\mu$L, 0.60 mmol) was added to the reaction mixture. The resulting solution was stirred at room temperature for 24 h. The solution was evaporated and the residue was dissolved in EtOAc (10 mL). The EtOAc solution was washed with 5% aq. citric acid (10 mL), 5% aq. $Na_2CO_3$ (10 mL), and brine (10 mL), and then dried over $Na_2SO_4$. The solvent was evaporated and column chromatography afforded mixture of (*S*,*S*) and (*R*,*S*)-10-[3-(2-{[4-hydroxy-1-(naphthalene-2-carbonyl)-pyrrolidine-2-carbonyl]-[(1-methyl-1H-indol-3-yl)-(1,1,3,3-tetramethyl-butylcarbamoyl)-methyl]-amino}-acetylamino)-propyl]-1,4,7,10-tetraaza-cyclodecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester (**3**) as a yellowish oil. $R_f$ 0.7 (MC/MeOH 10:1); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.3 (br, 1H), 8.1 (s, 1H), 7.8 (d, 2H), 7.7 (m, 2H), 7.6 (m, 2H), 7.4-7.27 (br, 5H), 6.34 (s, 1H), 5.62 (s, 1H), 4.61-4.53 (br, 2H), 4.34 (s, 2H), 3.89 (s, 3H), 3.76 (m, 1H), 3.65 (m, 2H), 3.6-3.30 (br, 14H), 2.72-2.58 (br, 6H), 2.18 (m, 2H), 1.78-1.68 (br, 4H), 1.46-1.36 (br, 33H), 1.04-0.98 (m, 9H); HRMS (FAB+): Calcd for $C_{48}H_{69}N_9O_5$: 852.1607. Found 852.1612 (M + H). Conversion of **3** to 4-hydroxy-1-(naphthalene-2-carbonyl)-pyrrolidine-2-carboxylic acid [(1-methyl-*1H*-indol-3-yl)-(1,1,3,3-tetramethyl-butylcarbamoyl)-methyl]-{[3-(1,4,7,10-tetraaza-cyclododec-1-yl)-propylcarbamoyl]-methyl}-amide (**4**) and conversion of **4** to **B** were carried out according to the procedure reported previously.[9]

**Measurements.** Distilled and deionized water was used for preparation of buffer solutions. Buffers (0.05 M) used for the kinetic measurements were acetate (pH 5), 2-(*N*-morpholino)ethanesulfonate (pH 6), *N*-2-hydroxyethylpiperazine-*N*′-ethanesulfonate (pH 7-8), and borate (pH 9.0). Buffer solutions were filtered with 0.45 $\mu$m Millipore microfilter and autoclaved before use in the kinetic measurements. Kinetic data were collected at 37 ± 0.1 °C. The concentration of MCH during the action of **B** was measured after tagging the *N*-terminal amino group of MCH with fluorescamine and quantifying the tagged MCH by HPLC as described previously.[9] NMR spectra were recorded with a Bruker DPX 300 MHz model. HPLC analysis was carried out with a 1100 series Agilent Technology HPLC.

## Results and Discussion

**Docking Simulations.** Figure 1 shows the lowest-energy conformation of **A** on the surface of MCH that was predicted from docking simulations. It is noted that the water ligand of Co(III) and the amide group in the central acyclic chain of **A** forms a bifurcated hydrogen bond with backbone amino-carbonyl oxygen of Phe(2) of MCH. A strong hydrogen bond is also established between the terminal hydroxyl moiety of **A** and the side chain of Arg(6). In this MCH-**A** complex, the *N*-terminal amino nitrogen resides in proximity of Co(III) at a distance of 3.61 Å, supporting the possibility that its coordination to Co(III) would be involved in the mechanism of the oxidative decarboxylation reaction. The three terminal groups of **A** form a hydrophobic pocket and point toward the side chains of Met(4) and Leu(5), indicating that van der Waals interactions between **A** and MCH should also play a role in stabilizing the protein-catalyst complex. Each dotted line indicates a hydrogen bond.

**Kinetic Data.** On the basis of the results of the docking simulation study, compound **B** was selected as the second catalyst. Here, the naphthoyl group replaces the acetyl group

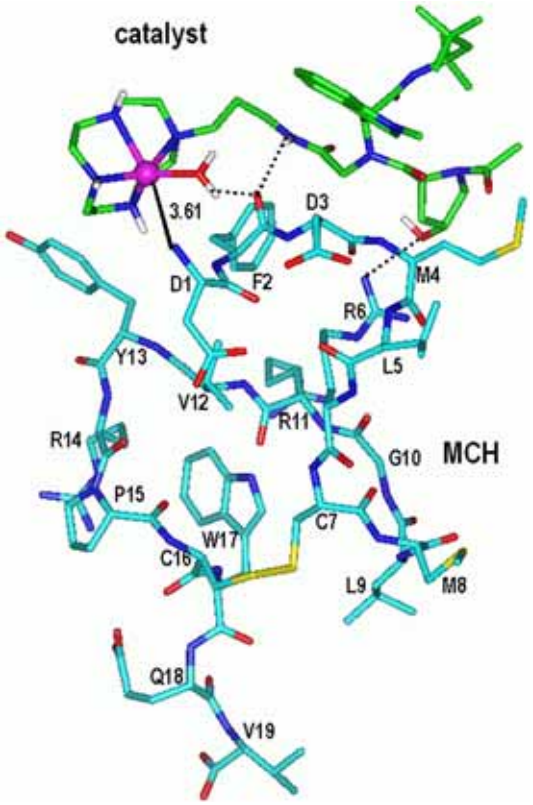



**Figure 1**. Predicted binding mode of **A** on the surface of MCH.

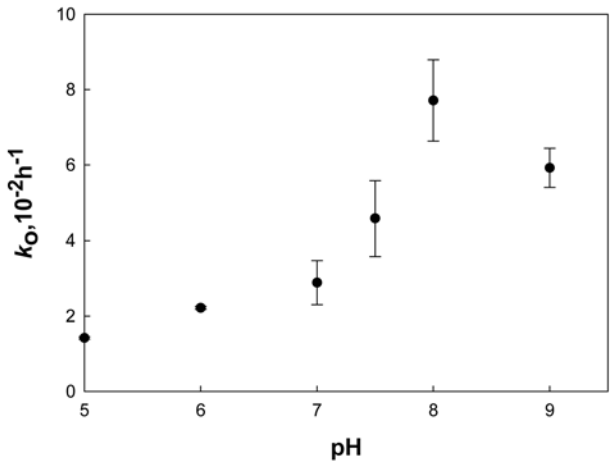



**Figure 2**. The pH profile of $k_o$ for cleavage of MCH by **B** at 37 °C ($S_o$ = 0.200 mM, $C_o$ = 1.00 mM).

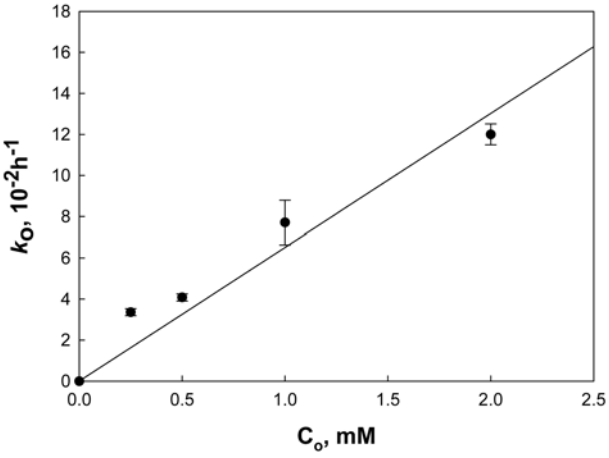



**Figure 3**. Plot of $k_o$ against $C_o$ ($S_o$ = 0.200 mM) for the disappearance of MCH by **B** at pH 8.0 and 37 °C.

of **A** to enhance the van der Waals interaction between the catalyst and MCH. Ugi condensation reaction was employed to synthesize **B**. Although Ugi condensation reaction produces a stereogenic center, no attempts were made to separate the resulting diastereomers of **B**. Instead, it was assumed that only half of **B** added to MCH possesses catalytic activity as revealed by the previous study.[9]

The rate data for the cleavage of MCH catalyzed by **B** were collected under the conditions of $C_o$ (the initially-added concentration of the catalyst) of 1.00 mM and $S_o$ (the initially-added concentration of the substrate) of 0.200 mM at various pH values. Here, $C_o$ was taken as a half of the initially added concentration of **B** since only one diastereomer would possess catalytic activity. When $[C] = C_o >> [CS]$, pseudo-first-order kinetic behavior is to be observed.[9] Since the concentration of MCH decreased slowly even in the absence of catalysts,[9] the background rate was subtracted to estimate pseudo-first-order rate constants ($k_o$). The pH dependence of $k_o$ measured at a fixed $C_o$ (Figure 2) showed that the optimum activity was manifested at pH 8.0. The same optimum pH was observed for the action of **A** on MCH.[9]

The dependence of $k_o$ and $C_o$ for the catalytic activity of **B** was measured at pH 8.0 and the results are illustrated in Figure 3. The kinetic data of Figure 3 can be analyzed in terms of the Michaelis-Menten scheme as performed previously[9] with the MCH cleavage catalyzed by **A**. The linear dependence is consistent with very weak complexation between MCH and **B** ($K_m >> S_o$). The proportionality constant estimated from the kinetic data can be taken as $k_{cat}/K_m$. The value of $k_{cat}/K_m$ is 65 ± 1 $h^{-1}M^{-1}$ at pH 8.0 for **B**. In the previous study, similar kinetic behavior was observed for the MCH cleavage catalyzed by **A**, for which $k_{cat}/K_m$ was 89 ± 1 $h^{-1}M^{-1}$ at the optimum pH of 8.0.[9] The activity of **B** is, therefore, 73% of **A**.

**Evaluation of Predicted Structure.** In an effort to explain the catalytic activity of **A** in oxidative decarboxylation of MCH and to predict the structure of a new catalyst with improved activity, the docking simulations were carried out for the complex formed between **A** and MCH. Since the experimental data for three dimensional structure of MCH are not reported and the potential parameters associated with Co(III) complex are not available, the accuracy of the results of the docking simulations may not be high. According to the docking simulations, the three terminal groups of **A** form a hydrophobic pocket and point toward the side chains of Met(4) and Leu(5), suggesting that van der Waals interactions between **A** and MCH could also play a role in stabilizing the MCH-**A** complex. On the basis of the docking simulations study, we designed **B** as the new catalyst hoping that the replacement of the acetyl group of **A** with the naphthoyl group might enhance the van der Waals interaction between the hydrophobic pocket of the catalyst and MCH.

The catalytic activity of **B** is about 73% of that of **A**. The lower catalytic activity of **B** indicates that it is not easy to predict the structure of an improved catalyst on the basis of the docking simulations study. Unlike many disease-related proteins, the solution structure of MCH is not available, making the theoretical analysis more difficult.

It is possible that introduction of the naphthoyl group may have resulted in formation of a stronger complex formed between the catalyst and MCH but that it may have located the Co(III) center of the catalyst in a less productive position in the complex. Since the values of $k_{cat}$ and $K_m$ were not estimated separately for the action of both **A** and **B**, it is not possible compare the ability of complex formation and catalytic turnover separately. It is not likely at this stage that docking simulations can predict effects of structural variation of the catalysts on $k_{cat}$ and $K_m$.

From chemical libraries containing about 19000 catalysts, we were able to identify only **A** as the catalyst for oxidative decarboxylation of MCH.[9] The kinetic data obtained with **B** indicate that replacement of the acetyl portion of **A** with naphthoyl group retains the catalytic activity despite the large difference in the size of the acyl groups. The computational and the kinetic data of the present study reveal that a chemical library containing a large number of variants of **A**

possessing various acyl groups in the hydroxyproline moiety may produce additional catalysts with considerably improved activity.

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