PREPARATION OF MOLYBDENUM(IV) COMPLEXES OF SEQUENTIAL POLYPEPTIDES CONSISTING OF GLYCINE AND L-CYSTEINE AND THEIR CATALYTIC ACTIVITY FOR THE ELECTRON TRANSFER REDUCTION OF ACETYLENE IN PROTIC MEDIA

NOBUKI OGUNI*, SHOGO SHIMAZU and AKIRA NAKAMURA

Department of Macromolecular Science, Faculty of Science, Osaka University, Toyonaka, Osaka 560 (Japan)

(Received December 1, 1982)

Summary

Molybdenum(IV) complexes of sequential polypeptides, $(Gly_2Cys)_n$ and $(Gly_3Cys)_m$ were prepared, and their catalytic activities examined in the electron transfer reduction (by $NaBH_4$) of acetylene. These molybdenum complexes had excellent catalytic activity in comparison with that of *N*-carbobenzyloxy-L-cysteine methyl ester.

Introduction

The active site of nitrogenase has been considered to be a mixed metal cluster coordinated with inorganic as well as organic sulfides [1]. Cysteine thiolate groups of the protein are proposed as important ligands controlling the remarkable reactivity of the enzyme toward dinitrogen [2]. As simple models of the nitrogenase action, we reported the reduction of acetylene and dinitrogen by catalysis of binary systems of the molybdenum complexes of L-cysteine-containing random polypeptide or *p*-mercaptomethyl-substituted polystyrene and ferredoxin model compounds, $[Fe_4S_4(SR)_4]^{2-}$ using $NaBH_4$ [3, 4]. It was found that these polymer–molybdenum complexes had activities remarkably higher than those of the corresponding unsupported molybdenum complexes. Random sequences of these polymers complicate the rational understanding of the catalysis, and further improvement of the activity is therefore expected by use of *sequential* polymers.

*Present address: Department of Chemistry, Faculty of Science, Yamaguchi University, Yoshida, Yamaguchi City, Yamaguchi, Japan 753.

0304-5102/83/$3.00

A peptide sequence, Cys—X—Y—Cys (X, Y = amino acid residue other than Cys), has been frequently found in various metalloproteins and is known to form macro-ring chelates with transition metal ions. We have chosen the sequences Cys—Gly—Gly—Cys and Cys—Gly—Gly—Gly—Cys in coordination polymers as models of nitrogenase, since these sequences are expected to accommodate heavy metal ions such as Mo through chelation.

This report deals with (a) the preparation of the molybdenum(IV) complexes of two kinds of *sequential* polypeptides containing L-cysteine residues, $(GlyCysGly)_n$ and $(GlyCysGly_2)_n$; (b) inferences on the characteristics of the coordination of polypeptides to Mo ions by means of CD spectroscopy; and (c) the catalytic behavior of the polypeptide–molybdenum/$[Fe_4S_4(SPh)_4]^{2-}$ systems for the reduction of acetylene.

Results and discussion

Preparation of catalyst

Sequential polypeptides containing *S*-benzylthiomethyl-L-cysteine (Cys(*S*-btm)) and glycine were prepared as follows;

$$Z\text{-GlyCys}(S\text{-btm})\text{Gly}_n\text{OH (I)} \xrightarrow{P(OC_6H_3Cl_2)_3\text{—pyridine}}$$

$$Z\text{-GlyCys}(S\text{-btm})\text{Gly}_n\text{OC}_6\text{H}_3\text{Cl}_2\text{ (II)} \xrightarrow{Et_3SiH/Pd\ catalyst}$$

$$ClH_3NGlyCys(S\text{-btm})\text{Gly}_n\text{OC}_6\text{H}_3\text{Cl}_2 \xrightarrow{1/4\ (II) + Et_3N}$$

$$Z\text{-[GlyCys}(S\text{-btm})\text{Gly}_n]_m\text{OC}_6\text{H}_3\text{Cl}_2\text{ (III)} \xrightarrow{t\text{-BuNH}_2}$$

$$Z\text{-[GlyCys}(S\text{-btm})\text{Gly}_n]_m\text{NHt-Bu (IV)}$$

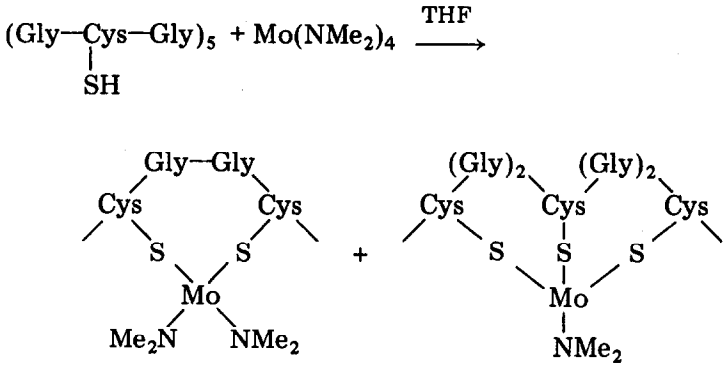
(n = 1 and 2, m = 5)

The tripeptide (n = 1) and the tetrapeptide (n = 2) (I) used in the preparation were synthesized by conventional methods [6]. Polymerization of these peptides was carried out with an activated ester method [7]. The degree of polymerization of the products, which was determined by ^{1}H NMR spectroscopy and liquid chromatography, was *ca.* 5.

In a previous report, we found that tetrakis(dimethylamido)–molybdenum(IV) was a useful catalyst precursor which reacted with thiols to give the thiolate complex of molybdenum with no change in formal oxidation state [4].

The polypeptide–molybdenum complexes were obtained by the reaction of polypeptides containing cysteine residues with $Mo(NMe_2)_4$ in THF under argon atmosphere in a molar ratio of 1:1 (thiol:Mo).

As the polypeptide chain contains about five cysteine thiolate groups on the average, chelate complexes were expected. We have compared the CD spectra of the polypeptide–molybdenum complexes with that of the *N,O*-blocked L-cysteine–molybdenum complex which has a monodentate cysteine ligand (Fig. 1). The polypeptide–molybdenum complex, $(GlyCysGly)_5$/Mo-$(NMe_2)_4$ had a negative peak at 358 nm, and on reduction exhibited a negative peak at 400 nm. The complex $(GlyCysGly_2)_5/Mo(NMe_2)_4$ had a negative peak at 380 nm which shifted on reduction with $NaBH_4$ to 403 nm, and a positive peak at 440 nm. The positions of the extrema were bathochromically shifted in each reduced system, but had similar Cotton effects to indicate that molybdenum–cysteine bonds were maintained on reduction. The CD spectrum of the system *Z*-CysOMe/$Mo(NMe_2)_4$ had a negative peak at 441 nm ($\Delta\epsilon = 1.79 \times 10^{-2}$) and a positive one at 487 nm ($\Delta\epsilon = 9.78 \times 10^{-3}$). The intensity of the CD spectra of the *Z*-CysOMe/molybdenum complex was about one fifth that of the polypeptide–molybdenum complexes chelated with thiolate groups of the polypeptide. Plausible structures of the complex were estimated based on the above CD spectra and on the molybdenum content in the complexes as follows:



Recently, CD and NMR spectra of the molybdenum complexes bearing tetrapeptides containing cysteine residues were investigated, and the macrocyclic chelate structure was confirmed for the corresponding Pd(II) and Fe(III) complexes [5].

Acetylene reduction by catalysis of binary systems of $Mo(NMe_2)_4$–Z-Cys-(OMe)/$[Fe_4S_4(SPh)_4]^{2-}$ and polypeptide–$Mo(NMe_2)_4/[Fe_4S_4(SPh)_4]^{2-}$ with $NaBH_4$ as reductant

One of characteristics of nitrogenase is its ability to reduce many substrates other than nitrogen, for example acetylene, cyanide, nitriles and nitrous oxide [8]. The reduction of acetylene with catalyst systems containing molybdenum complexes as mimics of nitrogenase has been studied.

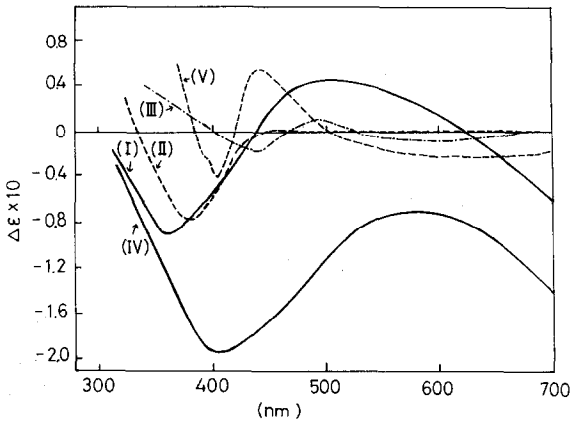


Fig. 1. CD spectra of sequential polypeptide–molybdenum(IV) complexes in DMF. Spectra (I), (II) and (III): complexes isolated by the reaction of $Mo(NMe_2)_4$ with $(GlyCysGly)_n$, $(GlyCysGly_2)_m$, and *Z*-CysOMe, respectively (ratio thiol: Mo = 2); spectra (IV) and (V): complexes of (I) and (II), respectively, reduced with $NaBH_4$ (ratio Mo: $NaBH_4$ = 1).

The results obtained in the catalytic reduction of acetylene with *Z*-Cys(OMe)— and polypeptide–$Mo(NMe_2)_4/[Fe_4S_4(SPh)_4]^{2-}$ systems are shown in Table 1, and the time–conversion curves of acetylene reduction are illustrated in Fig. 2. Very high activity of the polypeptide–molybdenum complex was observed in comparison with that of the *Z*-Cys(OMe)/$Mo(NMe_2)_4$ system. A similar tendency was already observed in catalytic systems of benzylmercaptan/$Mo(NMe_2)_4$ and partially *p*-mercaptomethyl-substituted polystyrene/$Mo(NMe_2)_4$ systems [4].

TABLE 1

Selectivity and activity in catalysis by molybdenum complexes for acetylene reduction by $NaBH_4$

Catalyst system	Time (h)	Conversion (%)	Product			Turnover number (mol/mol[Mo] min^{-1})
			ethylene (%)	butadiene (%)	ethane (%)	
$Mo(NMe_2)_4$	5	28.0	18.5	4.0	1.5	0.19
$Mo(NMe_2)_4$ + *Z*-L-Cys(OMe)	3	18.0	11.2	2.0	2.8	0.20
$Mo(NMe)_4$ + 2*Z*-L-Cys(OMe)	3	12.6	8.3	1.6	1.9	0.14
$Mo(NMe_2)_4$ + $(GlyCysGly)_5$	3	86.7	17.9	12.2	44.4	1.80
$Mo(NMe_2)_4$ + $(GlyCysGly_2)_5$	3	89.5	25.7	11.5	40.8	1.91
$Mo_2(NMe_2)_6$	3	3.6	3.0	0.3	0.3	0.04

Conditions: acetylene 3.93 mmol; $NaBH_4$ 7.9 mmol; catalyst 0.0197 mmol (Mo); Fe/Mo = 4; temperature 30 °C; solvent EtOH–THF–MeOH (2.5:1.5:1) (total 15.5 ml) under nitrogen.

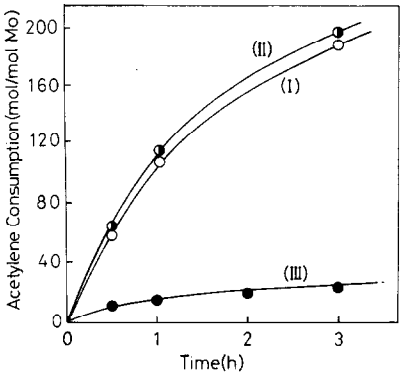


Fig. 2. Acetylene consumption by catalysis of a binary system composed of the sequential polypeptide–molybdenum complex–ferredoxin model compound, $[Fe_4S_4(SPh)_4]^{2-}$ $[N(n\text{-}Bu)_4]_2^{2+}$, with $NaBH_4$. Curves (I), (II) and (III): $Mo(NMe_2)_4$—$(GlyCysGly)_n$, —$(Gly\text{-}CysGly_2)_m$, and *Z*-CysOMe, respectively. Reaction conditions: acetylene, 15 mmol; molybdenum complexes (Mo) 1.97×10^{-2} mmol; ratio Fe: Mo = 4; solvent THF/EtOH, 4.6/7.4 ml; $NaBH_4$, 7.9 mmol; temperature 30 °C.

Formation of butadiene has been observed in the reduction of acetylene by catalysis of Mo(V)/cysteine or Mo(IV)/polymer [4]. In view of the recent report [10] of Chisholm *et al.* on formation of metallacyclopentadiene from $Mo_2(O—neoPent)_6$ and acetylene, the formation of butadiene may be understood by protolytic reaction of the metallacycle. Since the amount of butadiene has been correlated [4] with the extent of thiolate coordination around Mo(IV), the presence of *ca.* 2 thiolate ligands on the Mo species, inferred from the Mo content, is supported.

For comparison of the catalysis of Mo(IV)/Cys-containing copolypeptides in random sequence, two different copolypeptides of composition $(CysGlu(\gamma\text{-}Bz)_6)_n$ and $(CysGlu(\gamma\text{-}Bz)_9)_n$ were prepared [3]. Examination of catalysis using the random copolypeptides as ligands in experimental conditions similar to those in Table 1 indicated roughly 1/10 activity. The Glu residue thus seems to lower the activity. A Glu-containing peptide of the sequence $(Glu(\gamma\text{-}Bz)—Cys—Gly)_n$ was then examined as ligand to reveal similar low activity. Gly residue is thus superior to Glu(γ-Bz) residue in the component of Cys-containing peptides in the catalysis. The difference may be attributed to the enhanced conformational freedom of Gly in comparison with Glu.

Generally, Gly prefers to be one of the amino acid residues at the β-bend of proteins. Bending is a prerequisite for macro-ring chelation through coordination of side chain atoms; therefore, macro-ring chelation with the sequence Cys—Gly—Gly—Cys probably assists catalysis by keeping the coordination environment active against various deactivation pathways. One of them may be the formation of a Mo—Mo multiply-bonded species which is catalytically less active. Actually $(Me_2N)_3Mo{\equiv}Mo(NMe_2)_3$ had much lower catalytic activity than $Mo(NMe_2)_4$ [4]. Constraint of conformation imposed by the chelation of the polypeptide to molybdenum is then responsible for

the high catalytic activity in acetylene reduction. Our results support the high catalytic activity of nitrogenase which probably has a low-valent Mo species coordinated with the cysteine thiolate groups of peptides adopting relatively rigid conformations.

Experimental section

Instrumentation

Visible and UV spectra were taken with a JASCO-UNIDEC 5A spectrometer at room temperature. The CD was obtained by a JASCO-J40 spectropolarimeter in the region of 300 - 700 nm at room temperature. The identification and quantitative determination of the products in the reduction of acetylene were made using a YANACO gas chromatograph, Model G80 equipped with Porapak N as column packing material at 40 - 160 °C. NMR spectra were taken with a Varian XL-100A instrument.

Reagents

All amino acids in this work were the Peptide Institute's products. Tetrahydrofuran (THF), n-hexane, ether and petroleum ether were distilled from sodium benzophenone ketyl under argon atmosphere.

Synthesis of tetrakis(dimethylamido)molybdenum(IV)

This compound was prepared by the method reported by Bradley and Chisholm [9]. $Mo(NMe_2)_4$ (0.02 mol) was obtained by the reaction of $MoCl_5$ (0.22 mol) with $LiNMe_2$ (1.1 mol). The product was an air-sensitive purple solid, purified by distillation at 70 °C under 1×10^{-3} mmHg.

Synthesis of peptides, Z-GlyCys(S-btm)Gly (1) and Z-GlyCys(S-btm)Gly$_2$ (2)

These compounds were prepared by the step-wise method reported by Brownlee *et al.* [6]. Dicyclohexylcarbodiimide was used as a condensing agent. Overall yield, 38% for (1) and 32% for (2). i.e. $[\alpha]_D^{17} = +8.7°$ ($c = 0.5$ in $CHCl_3$) for (1) and +7.5° ($c = 0.5$ in $CHCl_3$) for (2). Elemental analysis calcd. for (1) $C_{23}H_{27}N_3O_6S_2$: C, 54.63%; H, 5.38%; N, 8.31%; found: C, 53.98%; H, 5.35%; N, 8.23%; calcd. for (2) $C_{25}H_{30}N_4O_7S_2$: C, 53.36%; H, 5.37%; N, 9.95%; found: C, 53.28%; H, 5.38%; N, 9.78%.

Synthesis of sequential polypeptides from 1 and 2

For the preparation of sequential oligopeptides of 1 and 2, the method of active ester 2,4,5-trichlorophenyl ester was applied. To a solution of the peptide 1 or 2 (0.01 mol) in CH_2Cl_2 (40 ml), tris(2,4,5-trichlorophenyl) phosphite (0.005 mol) and pyridine (10 ml) were added slowly and the mixture was stirred for 10 h at room temperature. The solution was washed with dilute HCl and dilute $NaHCO_3$ aqueous solutions and dried over anhydrous Na_2SO_4. To the above solution, 4 drops of triethylamine and $PdCl_2$ (50 mg) and triethylsilane (1.7 g) in THF (20 ml) were added and the mixture

was refluxed for 3 h [11]. After removal of catalyst by filtration, methanol (10 ml) saturated with HCl gas and then acetone (30 ml) were added in order to precipitate the *N*-deblocked peptide hydrogen chloride. The *N*-deblocked peptide (1.4 mmol) and the peptide 1 or 2 (0.35 mmol) were dissolved in THF (40 ml) at 20 °C under argon. Condensation of these peptides was performed by addition of triethylamine (1.4 mmol). The solution was stirred for 5 h at 20 °C and then the mixture was filtered to remove triethylammonium chloride. Hexane (100 ml) was added to the filtrate to obtain the sequential polypeptide. The crude polymer was dissolved in THF and reacted with t-butylamine to remove trichlorophenyl ester groups from the end group of the polypeptide. Then the polypeptide was separated fractionally through column chromatography (Alumina Activated 300, solvent $CHCl_3$). The degree of polymerization of the main fraction was *ca.* 5.0, which was determined by proton ratio in the ^1H NMR spectrum [t-butyl(δ1.2)/phenyl(δ7.2)]. For the polypeptide from 1 m.p. 206 - 210 °, $[\alpha]_D^{18} = -10.5°$ (c = 0.2 in DMF). For the polypeptide from 2 m.p. 208 - 212 °, $[\alpha]_D^{16.5} = -5.4°$ (c = 0.2 in DMF).

Synthesis of a sequential peptide [Glu(γ-Bz)—Cys(S-btm)—Gly]$_n$

A protected dipeptide, [(BzGlu)(γ-Bz)—Cys(*S*-btm)][NH_2(c-Hex)$_2$], was prepared in a manner similar to that of Brownlee *et al.* [6] from [BzGlu-(γ-Bz)][NH_2N(c-Hex)$_2$] and HCl·Cys(*S*-btm)OCH_3 in 47% yield. $[\alpha]_D^{22} = -20.7°$ (c = 0.58 in $CHCl_3$). The dipeptide was coupled with HCl·GlyOEt by DCC. The ester portion was hydrolyzed to the corresponding acid, which was then esterified by P($OC_6H_4NO_2$)$_3$ to give BzGlu(γ-Bz)—Cys(*S*-btm)—Gly—$OC_6H_4NO_2$ (3) in 49% yield, $[\alpha]_D^{17} = -33.2°$ (c = 0.53 in $CHCl_3$). The Bz group at the *N*-terminal was cleaved by $Et_3SiH/PdCl_2$ as described above; the product (4) was polymerized with 3 by NEt_3 in THF at 20 °C for 5 h, and terminated by addition of t-BuNH_2 followed by separation through silica gel column chromatography to give the sequential peptide with n = 3.4 as determined by elemental analysis and ^1H NMR spectra.

Preparation of sequential polypeptide–molybdenum complexes

The sequential polypeptide (1.0 g) was dissolved in dichloroacetic acid (12.5 ml), and a partial suspension of mercuric acetate (four equivalents/cysteine residues) in water was added with stirring under nitrogen to give a clear solution. After 1 h of stirring at room temperature, ethanedithiol (2.5 equivalents/mercuric acetate) was added to precipitate mercuric sulfide. After removal of mercuric sulfide by filtration, *S*-deblocked polypeptide was obtained as precipitate by addition of ether which was previously degassed *in vacuo*. The resulting polypeptide was dried *in vacuo*. For poly(Gly—L-Cys—Gly): $[\alpha]_D^{18.0} = -2.64°$ (c = 0.2 in DMF). For poly(Gly—L-Cys—Gly_2): $[\alpha]_D^{18.0} = -7.53°$ (c = 0.2 in DMF). A mixture of Mo(NMe_2)$_4$ (2 equivalents/cysteine residues) and *S*-deblocked polypeptide (0.1 g) in THF (50 ml) under argon was stirred for 24 h at room temperature. Ether degassed previously *in vacuo* was added to obtain the polypeptide–molybdenum complex, which was then separated by filtration and washed with cyclohexane under argon in order to remove unreacted Mo(NMe_2)$_4$.

Reduction of acetylene by catalysis of a binary system of polypeptide/molybdenum complex and $[Fe_4S_4(SPh)_4]^{2-}$

The polypeptide/molybdenum complex described above and $[N(n\text{-}Bu)_4]_2[Fe_4S_4(SPh)_4]$ (0.0197 mmol) were mixed in THF (4.6 ml) under argon. Then $NaBH_4$ (7.9 mmol) was added to the above catalyst solution. After replacing argon in the reaction vessel with acetylene, ethanol (7.4 ml) and methanol (3.0 ml) were added to the above mixture, and the solution was stirred vigorously in a water bath kept at 30 °C. The products in the vapor phase and in solution (removed by syringes) were quantitatively analyzed by gas chromatography at specific time intervals.

References

1 S. P. Cramer, K. O. Hodgson, W. D. Gillum and L. E. Mortenson, *J. Am. Chem. Soc., 100* (1978) 3398.
2 P. J. Stephens, C. E. McKenna, M. C. McKenna, H. T. Nguyen and F. Devlin, *Biochemistry, 20* (1981) 2857.
3 N. Oguni, S. Shimazu and A. Nakamura, *Polymer J., 12* (1980) 891.
4 N. Oguni, S. Shimazu, Y. Iwamoto and A. Nakamura, *Polymer J., 13* (1981) 845.
5 N. Ueyama, M. Nakata and A. Nakamura, *Inorg. Chim. Acta, 55* (1981) L-61; *Bull. Chem. Soc. Jpn., 54* (1981) 1727.
6 P. J. E. Brownlee, M. E. Cox, B. O. Handford, J. C. Marsden and G. T. Young, *J. Chem. Soc.*, (1964) 3832.
7 J. Pless and R. A. Boissonnas, *Helv. Chim. Acta, 46* (1963) 1609.
8 B. J. Weathers, J. H. Grate, N. A. Strampach and G. N. Schrauzer, *J. Am. Chem. Soc., 101* (1979) 925.
9 D. C. Bradley and M. H. Chisholm, *J. Chem. Soc., (A)* (1971) 2741.
10 M. H. Chisholm, K. Folting, J. C. Huffman and I. P. Rothwell, *J. Am. Chem. Soc., 104* (1982) 4389.
11 L. Birkhofer, E. Bierwirth and A. Ritter, *Chem. Ber., 94* (1961) 821.