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Synthesis of Two Heptapeptides corresponding to Sequences 50—56 and 90—96 of Adrenodoxin from Bovine Adrenal Cortex and Formation of Their Iron-Sulfur Complexes[1]

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Two heptapeptides, Z–Leu–Ala–Cys(Bzl)–Ser–Thr–Cys(Bzl)–His–OH (A) and Z–Leu–Gly–Cys(Bzl)–Gln–Ile–Cys(Bzl)–Leu–OH (B), corresponding to sequences 50—56 and 90—96 of adrenodoxin from bovine adrenal cortex, respectively, were synthesized. The deblocked peptides from A and B formed iron-sulfur complexes, both of which gave absorption maxima at 415 nm.

Keywords——synthesis of heptapeptides; partial structure of adrenodoxin; Na in liquid ammonia; iron-sulfur complex; absorption spectra

Adrenodoxin, which contains two iron atoms and two moles of labile sulfide per mole of protein,[3] is a component of the electron transport system in the adrenal cortex which hydroxylates steroids.[4–6] Tanaka *et al.*[7] reported the entire amino acid sequence of bovine adrenodoxin, which consists of 114 amino acid residues. They postulated that four cysteine residues at positions 52, 55, 92 and 95 out of the total of five cysteine residues participate in the formation of the chelate structure of adrenodoxin, as shown in Fig. 1. The structure, Cys–X–Y–Cys (X, Y= amino acids) is observed in other non-heme electron transferring enzymes, and it is well known that these cysteine residues contribute to the binding with iron atoms.

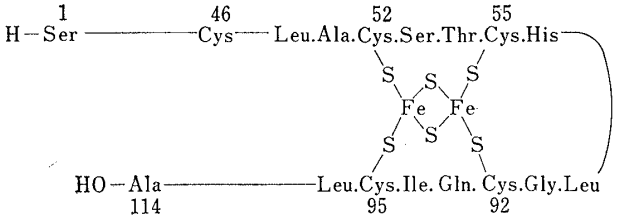

Fig. 1. Possible Chelate Structure of Bovine Adrenodoxin

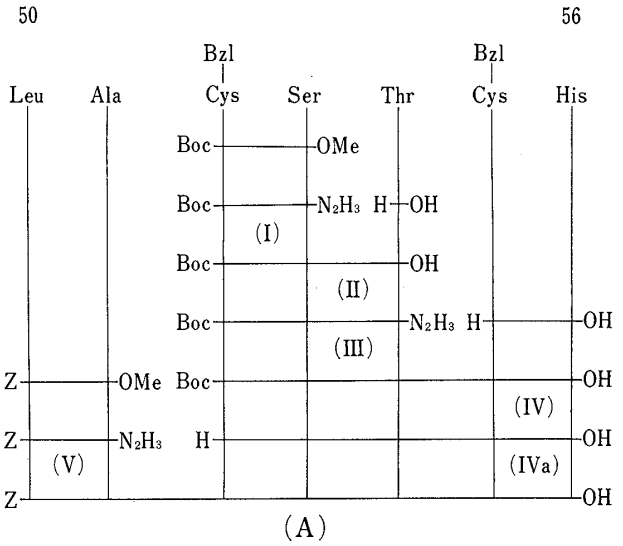

Fig. 2. Synthetic Scheme for the Protected Heptapeptide (A)

1) Amino acids, peptides and their derivatives mentioned in this paper are of the L-configuration. Abbreviations used are those recommended by the IUPAC-IUB commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl, *t*-Boc= *tert*-butyloxycarbonyl, Bzl=benzyl.
2) Location: *Ikawadani-machi, Tarumi-ku, Kobe, 673 Japan.*
3) T. Kimura and K. Suzuki, *J. Biol. Chem.*, **242**, 485 (1967).
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Sugiura *et al.*[8] previously obtained an absorption spectrum similar to that of native ferredoxin from green plants by mixing ferric chloride and sodium sulfide with the pentapeptide, H–Ser–Cys–Val–Ser–Cys–OH,[9] which is related to the ferredoxin from *Clostridium pasteurianum*. Ali *et al.*[10] reported the synthesis of a cysteine-containing model decapeptide related to rubredoxin from *Micrococcus aerogenes* and formation of the iron complex by mixing ferrous ammonium sulfate with the synthetic peptide. The color of the complex was similar to that of native rubredoxin.

These results suggest that appropriate peptides which contain cysteine residues may form chelate structures with iron-sulfur or iron atoms which are similar to the active cores of non-heme electron transferring substances such as adrenodoxin, ferredoxin and rubredoxin. Thus, we undertook the synthesis of two heptapeptides, Z–Leu–Ala–Cys(Bzl)–Ser–Thr–Cys-(Bzl)–His–OH (A) and Z–Leu–Gly–Cys(Bzl)–Gln–Ile–Cys(Bzl)–Leu–OH (B) which correspond to sequences 50—56 and 90—96 of bovine adrenodoxin, respectively. The present report describes the synthesis of the two heptapeptides, A and B, and the formation of iron-sulfur complexes of the deblocked peptides.

The synthetic scheme for A is illustrated in Fig. 2. The benzyl group, removable on exposure to sodium in liquid ammonia,[11] was selected as a protecting group for the sulfhydryl group of cysteine. The synthesis of the protected heptapeptide (A) was accomplished by azide coupling[12,13] of three fragments as follows. *t*-Boc–Cys(Bzl)–Ser–OMe[14] was converted to the corresponding hydrazide (I), which was linked to the triethylammonium salt of H–Thr–OH by the azide condensation method to afford *t*-Boc–Cys(Bzl)–Ser–Thr–OH (II). II was converted to the methyl ester with diazomethane and further to the corresponding hydrazide (III) by treatment with hydrazine hydrate in methanol. III was coupled with H–Cys(Bzl)–His–OH[15] by the azide procedure to give *t*-Boc–Cys(Bzl)–Ser–Thr–Cys(Bzl)–His–OH (IV). Removal of the *t*-Boc group of IV was performed by trifluoroacetic acid (TFA) treatment in the usual manner to afford H–Cys(Bzl)–Ser–Thr–Cys(Bzl)–His–OH (IVa). A *t*-Boc group[16] on the amino group was used at this stage to avoid acetylation of the hydroxyl group of serine[17,18] on treatment of the serine-containing peptide with hydrogen bromide in acetic acid. IVa was condensed with Z–Leu–Ala–$NHNH_2$ (V) by the azide procedure to afford Z–Leu–Ala–Cys(Bzl)–Ser–Thr–Cys(Bzl)–His–OH (A).

The Synthetic route to B is illustrated in Fig. 3. The C-terminal pentapeptide, Z–Cys-(Bzl)–Gln–Ile–Cys(Bzl)–Leu–OH (X) was constructed in a stepwise manner starting from H–Leu–OMe[19] and using N-benzyloxycarbonyl amino acids, exclusively by the mixed anhydride method.[20] Z–Cys(Bzl)–OH[21,22] and H–Leu–OMe were coupled to yield Z–Cys(Bzl)–Leu–OMe (VI). Treatment of VI with hydrogen bromide in acetic acid gave the corresponding hydrobromide salt (VIa), which was coupled with Z–Ile–OH[23] to afford Z–Ile–Cys(Bzl)–Leu–OMe (VII). VII was saponified with sodium hydroxide in dioxane to form the corresponding

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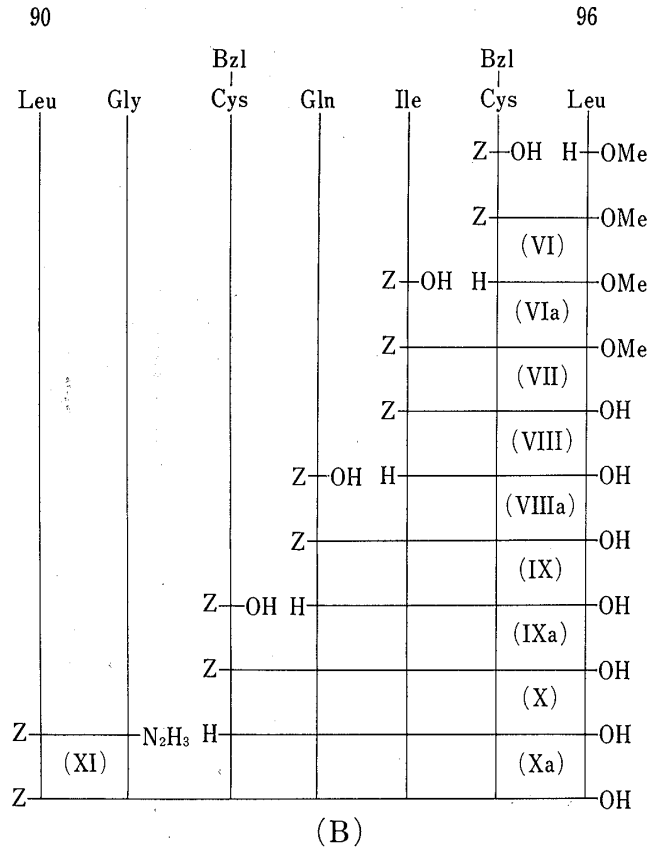

Fig. 3. Synthetic Scheme for the Protected Heptapeptide (B)

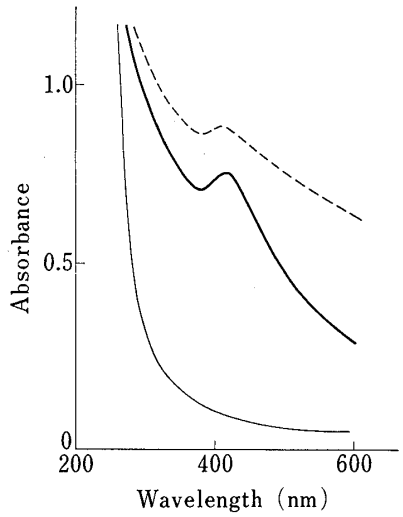

Fig. 4. Absorption Spectra of Iron-Sulfur Complexes of the Deblocked Peptides from A and B in 10 mM Tris Buffer (pH 7.5)

——, deblocked A (50 mg, 0.047 mM), mercaptoethanol (73 mg, 0.94 mM), Na_2S (0.35 mM) and $FeCl_3$ (0.24 mM);
——, deblocked B (50 mg, 0.047 mM), mercaptoethanol (73 mg, 0.94 mM), Na_2S (0.35 mM) and $FeCl_3$ (0.24 mM);
——, mercaptoethanol (73 mg, 0.94 mM), Na_2S (0.35 mM) and $FeCl_3$ (0.24 mM).

tripeptide acid (VIII), which after removal of the N-protecting group with hydrogen bromide in acetic acid, was coupled with Z–Gln–OH[23] to give Z–Gln–Ile–Cys(Bzl)–Leu–OH (IX). IX was converted to the corresponding tetrapeptide amine hydrobromide (IXa) by treatment with hydrogen bromide in acetic acid, and Z–Cys(Bzl)–OH was coupled with IXa to give Z–Cys(Bzl)–Gln–Ile–Cys(Bzl)–Leu–OH (X). X, after deblocking at the amino end, was coupled with Z–Leu–Gly–$NHNH_2$ (XI) by the azide coupling method to afford Z–Leu–Gly–Cys(Bzl)–Gln–Ile–Cys(Bzl)–Leu–OH (B).

To check the optical purity of the constituent amino acids of A and B, the protected heptapeptides were converted to the corresponding S-sulfonated heptapeptides by treatment with sodium in liquid ammonia, followed by oxidative sulfitolysis[24] at pH 8 and purification by repeated gel-filtration on Sephadex G-15, then the resulting S-sulfonated peptides were digested with aminopeptidase M (AP-M).[25] Amino acid analysis of the digests as well as acid hydrolysates gave molar ratios in a very good agreement with the theoretically expected values. We can conclude that the S-sulfonated heptapeptides derived from A and B were completely digested by the enzyme, indicating that the configuration of the constituent amino acids was preserved during the synthetic process.

Formation of an iron-sulfur complex with deblocked A or B was carried out according to the procedure reported for the reconstitution of modified adrenodoxins from modified apoadreodoxins.[26] Protected heptapeptide (A) or (B) was treated with sodium in liquid ammonia[11] to remove the benzyloxycarbonyl group and S-benzyl group on the cysteine residues. After removal of ammonia, the residue was mixed at 0° with Na_2S and $FeCl_3$ in

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10 mM Tris buffer (pH 7.5) containing mercaptoethanol. After 2 hr, the mixture was centrifuged. Absorption spectra of the brown-colored supernatant, which contained the complex of deblocked A or B, are shown in Fig. 4. It was previously reported that oxidized native adrenodoxin had absorption maxima at 455 nm, 414 nm and 320 nm (broad).[3] However, the absorption spectrum of the complexes of the peptides obtained above showed only a single maximum at 415 nm. This peak was still observed after 24 hr at 0°, although the absorbance had decreased slightly.

Sugiura *et al.*[8] investigated complexes formed from many kinds of sulfur-containing ligands with iron, or iron and sulfur atoms. They reported that a peak around 415 nm appeared on the formation of iron-sulfur complexes from iron complexes. Taniguchi *et al.*[26] reconstituted modified adrenodoxins from modified apoadrenodoxins, iron and sulfur atoms. Each modified adrenodoxin exhibited a peak at around 414 nm and a slight shoulder around 450 nm which are due to the iron-sulfur chromophore. In addition, they discussed the stability of the iron-sulfur center in the modified adrenodoxins by following the absorbance at 414 nm as a function of time. From their data and our results presented here, we can deduce that the brown-colored complexes obtained above are formed from the peptides with iron and sulfur atoms, as in the cases of ferredoxin and adrenodoxin, and that the iron-sulfur center of the complexes is quite stable at 0°.

Elongation of the peptides, A and B, is under way in our laboratory to investigate the possibility of forming an active core of non-heme iron-sulfur electron transferring enzymes.

Experimental

The melting points are uncorrected. Optical rotations were measured with an automatic polarimeter, model DIP-180 (Japan Spectroscopic Co., Ltd.). Amino acid compositions of acid and enzymatic hydrolysates were determined with a JEOL JLC-6AH amino acid analyzer. Absorption spectra were recorded with a Hitachi 323 recording spectrophotometer. Solvents were evaporated off *in vacuo* at a bath temperature of 40—50° in a rotary evaporator. For column chromatography, a Toyo SF-160K fraction collector was used. On thin-layer chromatography (Kieselgel G, Merck), Rf^1 and Rf^2 values refer to the systems of *n*-butanol, AcOH and H_2O (4: 1: 5), and *n*-butanol, pyridine, AcOH and H_2O (4: 1: 1: 2), respectively.

***t*-Boc-Cys(Bzl)-Ser-NHNH$_2$ (I)**——Hydrazine hydrate (80%, 4 ml) was added to a solution of *t*-Boc-Cys(Bzl)-Ser-OMe[14] (3.2 g) in MeOH (30 ml). After 24 hr at room temperature, the solution was concentrated to a small volume. Upon addition of water, the product crystallized out. It was collected by filtration and recrystallized from MeOH and ether; yield 2.8 g (88%), mp 148—150°, $[\alpha]_D^{23}$ −13.6° (c=1, DMF). *Anal.* Calcd. for $C_{18}H_{28}N_4O_5S$: C, 52.4; H, 6.84; N, 13.6. Found: C, 52.7; H, 6.80; N, 13.4.

***t*-Boc-Cys(Bzl)-Ser-Thr-OH (II)**——The entire operation was carried out in a cold room at 4°. 1N HCl in DMF (12 ml) followed by isoamylnitrite (0.81 ml) was added to a solution of I (2.43 g) in DMF (15 ml) cooled to −15°. After 5 min, triethylamine (1.68 ml) was added. This solution was added to a solution of H-Thr-OH (0.81 g) and triethylamine (2.24 ml) in 40% aqueous DMF (18 ml). The reaction mixture was stirred for 48 hr at 4° and evaporated to dryness. The residue was extracted in 5% Na_2CO_3, which was washed twice with AcOEt. The water layer was acidified with 10% citric acid under cooling and oily precipitate was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to form a solid mass; yield 2.0 g (57%), mp 145° (dec.), $[\alpha]_D^{23}$ −9.6° (c=1, MeOH). *Anal.* Calcd. for $C_{22}H_{33}N_3O_8S$: C, 52.9; H, 6.66; N, 8.4. Found: C, 52.7; H, 6.75; N, 8.2.

***t*-Boc-Cys(Bzl)-Ser-Thr-NHNH$_2$ (III)**——An ethereal solution of diazomethane (prepared from 3 g of nitrosomethyl urea) was added to a solution of II (0.63 g) in MeOH (30 ml). The solution was stored for 2 hr at 2° and the solvent was removed by evaporation. The oily residue was dissolved in MeOH (10 ml) and 80% hydrazine hydrate (0.80 ml) was added. After 24 hr at room temperature, the white crystalline precipitate was collected and recrystallized from MeOH; yield 0.57 g (88%), mp 174—175° (dec.), $[\alpha]_D^{23}$ −2.7° (c=1, DMF). *Anal.* Calcd. for $C_{22}H_{35}N_5O_7S$: C, 51.5; H, 6.87; N, 13.6. Found: C, 51.5; H, 6.84; N, 13.6.

***t*-Boc-Cys(Bzl)-Ser-Thr-Cys(Bzl)-His-OH (IV)**——The entire operation was carried out in a cold room at 4°. Under cooling with ice-salt, III (0.40 g) was dissolved in 0.2N HCl in DMF (9 ml) and isoamylnitrite (0.11 ml) was added. After stirring for 5 min, triethylamine (0.23 ml) was added. This solution was added to a solution of H-Cys(Bzl)-His-OH[15] (prepared as follows: 0.39 g of Z-Cys(Bzl)-His-OH was dissolved in 3 ml of 25% HBr in acetic acid, then after 1 hr, ether was added and the precipitate was collected, washed with ether and dried over KOH pellets) and triethylamine (0.34 ml) in DMF (10 ml), and the reaction mixture was stirred for 48 hr. The solvent was removed by evaporation and the residue was dissolved in 5% aqueous

Na_2CO_3 (10 ml). The solution was washed twice with AcOEt. The water layer was acidified with glacial acetic acid to pH 6, and the oily precipitate was extracted with AcOEt. The extract was washed with water and condensed to a small volume to give a gelatinous precipitate. The product was collected by filtration; yield 0.32 g (48%), mp 110° (dec.), $[\alpha]_D^{23}$ −17.7° (c=1, MeOH). *Anal.* Calcd. for $C_{35}H_{51}N_7O_{10}S_2 \cdot H_2O$: C, 53.8; H, 6.30; N, 11.6. Found: C, 53.5; H, 6.42; N, 11.6.

Z-Leu-Ala-NHNH$_2$ (V)——Hydrazine hydrate (80%, 6 ml) was added to a solution of Z-Leu-Ala-OMe[27] (3.5 g) in MeOH (30 ml). The solution was kept at room temperature for 15 hr and condensed to form a white crystalline material, which was collected by filtration and recrystallized from MeOH and ether; yield 2.0 g (57%), mp 160—162°, $[\alpha]_D^{23}$ −8.6° (c=1, DMF). *Anal.* Calcd. for $C_{17}H_{26}N_4O_4$: C, 58.3; H, 7.48; N, 16.0. Found: C, 58.0; H, 7.32; N, 15.9.

Z-Leu-Ala-Cys(Bzl)-Ser-Thr-Cys(Bzl)-His-OH (A)——A solution of IV (0.2 g) in TFA (1.5 ml) containing anisole (0.1 ml) was stored at room temperature for 1 hr. On addition of ether (30 ml), the partially deblocked pentapeptide (IVa) was precipitated. It was collected by filtration, washed with ether and dried over KOH pellets *in vacuo*. This product was dissolved in DMF (8 ml), cooled to 0°, and then neutralized with triethylamine (0.08 ml). To this solution, the dipeptide azide prepared as follows was added. Isoamylnitrite (0.04 ml) was added to a solution of V (0.11 g) in 0.3 N HCl in DMF (2.3 ml) cooled to −15°. After 5 min, triethylamine (0.084 ml) was added and the solution was mixed with the pentapeptide (IVa) solution prepared above. The reaction mixture was stirred for 48 hr at 4° and the solvent was removed by evaporation. AcOEt (20 ml) was added to the residue and the precipitated product was collected by filtration, washed thoroughly with 10% citric acid and recrystallized from MeOH and AcOEt; yield 0.20 g (79%), mp 197—198° (dec.), $[\alpha]_D^{23}$ −29.9° (c=1, DMF), Rf^1 0.82. *Anal.* Calcd. for $C_{50}H_{65}N_9O_{12}S_2 \cdot 2H_2O$: C, 55.4; H, 6.41; N, 11.6. Found: C, 55.1; H, 6.49; N, 11.4. Amino acid ratios in an acid hydrolysate; Leu 1.0; Ala 0.9; Ser 0.8; Thr 0.9; His 1.1; Cys was not determined (average recovery 87%).

To check the optical purity of the constituent amino acids of A, H-Leu-Ala-Cys(SO_3^-)-Ser-Thr-Cys(SO_3^-)-His-OH was derived as follows. To a solution of A (70 mg) in liquid ammonia (100 ml), small pieces of Na were added at the boiling point of ammonia until the blue color remained for 30 sec. Ammonia was removed completely, the residue was dissolved in 8 M guanidine hydrochloride (5 ml), and the pH of the solution was adjusted to 8 with dilute NH_4OH. Sodium sulfite (680 mg) and freshly prepared sodium tetrathionate (320 mg) were added to this solution. The reaction mixture was stirred at room temperature for 18 hr and applied to a Sephadex G-15 column (1.0×170 cm) equilibrated with 5% AcOH. The column was eluted with 5% AcOH at a flow rate of 24 ml/hr, and fractions (2 ml each) were collected. Lyophilization of the effluent (fractions 49—57) afforded the desired product, which was rechromatographed in the same way to give purified S-sulfonated peptide; yield 52 mg (89%), Rf^2 0.30 (ninhydrin and Pauly stain). Amino acid ratios in an acid hydrolysate; Leu 1.0; Ala 1.0; Ser 0.8; Thr 0.7; His 1.0 (average recovery 80%) and amino acid ratios in an APM digest; Leu 1.0 Ala 1.0 Ser 0.9; Thr 1.0; His 0.9; S-sulfoCys 1.8 (average recovery 80%).

Z-Cys(Bzl)-Leu-OMe (VI)——A mixed anhydride was prepared from Z-Cys(Bzl)-OH (3.46 g) with triethylamine (1.38 ml) and ethyl chloroformate (0.96 ml) at −10° in tetrahydrofuran (THF) (25 ml), and this was added to an ice-cold solution of H-Leu-OMe (1.45 g) in THF (15 ml). After stirring in an ice bath for 3 hr, the solvent was removed and the residue was dissolved in ether. The solution was washed successively with 0.2 N HCl, 5% Na_2CO_3 and H_2O, then dried over Na_2SO_4. Removal of the solvent by evaporation gave a syrupy material, which was crystallized from petroleum ether; yield 3.4 g (72%), mp 92—94°, $[\alpha]_D^{23}$ −7.2° (c=1, MeOH). *Anal.* Calcd. for $C_{25}H_{32}N_2O_5S$: C, 63.5; H, 6.8; N, 5.9. Found: C, 63.2; H, 6.7; N, 6.0.

Z-Ile-Cys(Bzl)-Leu-OMe (VII)——VI (4.4 g) was treated with 25% HBr/AcOH (5 ml) in the usual manner. The resulting amine (VIa) was dissolved in dioxane (20 ml) and the pH of the solution was adjusted to 8 with triethylamine. A mixed anhydride prepared from Z-Ile-OH (2.65 g) with triethylamine (1.38 ml) and ethyl chloroformate (0.96 ml) was added to the dioxane solution of VIa prepared above. The mixture was stirred at 0° for 3 hr and evaporated down to a small volume to give a white crystalline material, which was collected by filtration, washed with 0.1 N HCl and ether, and recrystallized from MeOH; yield 4.7 g (80%), mp 179—180°, $[\alpha]_D^{23}$ −69.6° (c=1, MeOH). *Anal.* Calcd. for $C_{31}H_{43}N_3O_6S$: C, 63.6; H, 7.4; N, 7.2. Found: C, 63.7; H, 7.5; N, 7.0.

Z-Ile-Cys(Bzl)-Leu-OH (VIII)——A solution of VII (2.0 g) in dioxane (35 ml) and 1 N NaOH (3.4 ml) was stirred at room temperature for 40 min. After the addition of AcOH (1 ml), the solvent was evaporated off. The residual solid material was washed with 0.1 N HCl, and recrystallized from MeOH; yield 1.84 g (94%), mp 149—153°, $[\alpha]_D^{23}$ −32.6° (c=1, DMF). *Anal.* Calcd. for $C_{30}H_{41}N_3O_6S \cdot 1/2H_2O$: C, 62.0; H, 7.3; N, 7.3. Found: C, 62.0; H, 7.1; N, 7.5.

Z-Gln-Ile-Cys(Bzl)-Leu-OH (IX)——H-Ile-Cys(Bzl)-Leu-OH (prepared from 2 g of VIII and 2 ml of 25% HBr/AcOH in the presence of 0.4 ml of anisole) was dissolved in dioxane (7 ml) and the pH of the solution was adjusted to 8 with triethylamine. To this solution, a mixed anhydride prepared from Z-Gln-OH

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(1.54 g) with triethylamine (0.76 ml) and ethyl chloroformate (0.53 ml) in THF (50 ml) at −10° was added. The reaction mixture was stirred at 0° for 3 hr and the solvent was removed by evaporation to form a crystalline material, which was collected by filtration and washed with 0.1 N HCl, H_2O and MeOH; yield 1.81 g (74%), mp 230—235°, $[\alpha]_D^{23}$ −41.0° (c=1, AcOH). *Anal.* Calcd. for $C_{35}H_{49}N_5O_8S$: C, 60.1; H, 7.1; N, 10.0. Found: C, 60.3; H, 7.4; N, 10.2.

Z-Cys(Bzl)-Gln-Ile-Cys(Bzl)-Leu-OH (X)——H–Gln–Ile–Cys(Bzl)–Leu–OH (prepared from 1.6 g of IX with 2 ml of 25%HBr/AcOH in the presence of 0.5 ml of anisole) was dissolved in DMF (15 ml) and the pH of the solution was adjusted to 8 with triethylamine. A mixed anhydride prepared from Z–Cys(Bzl)–OH (1.39 g) in the manner described previously was added. The mixture was stirred at 0° for 3 hr and evaporated down to a small volume to give a solid material, which was collected by filtration, washed with 0.1 N HCl, dried and recrystallized from DMF and AcOEt; yield 1.44 g (70%), mp 235—238°, $[\alpha]_D^{23}$ −57.5° (c=1, AcOH). *Anal.* Calcd. for $C_{45}H_{60}N_6O_9S_2$: C, 60.5; H, 6.8; N, 9.4. Found: C, 60.2; H, 7.0; N, 9.6.

Z-Leu-Gly-NHNH$_2$ (XI)——Hydrazine hydrate (80%, 1 ml) was added to a solution of Z–Leu–Gly–OMe[28] (3.2 g) in MeOH (20 ml), and the mixture was kept at room temperature overnight. The solution was evaporated down to a small volume and ether was added to form a precipitate, which was collected by filtration and recrystallized from EtOH and ether; yield 2.9 g (90%), mp 125—127°, $[\alpha]_D^{23}$ −7.2° (c=1, MeOH). *Anal.* Calcd. for $C_{16}H_{24}N_4O_4$: C, 57.2; H, 7.1; N, 16.7. Found: C, 56.9; H, 7.3; N, 16.8.

Z-Leu-Gly-Cys(Bzl)-Gln-Ile-Cys(Bzl)-Leu-OH (B)——H–Cys(Bzl)–Gln–Ile–Cys(Bzl)–Leu–OH (Xa) (prepared from 1.3 g of X and 3 ml of 25% HBr/AcOH with 0.5 ml of anisole) was dissolved in DMF (5 ml) and the pH of the solution was adjusted to 8 with triethylamine. Z–Leu–Gly–N_3 (prepared from 0.81 g of XI as described in the synthesis of A) was added to this cold solution. The reaction mixture was stirred at 4° for 48 hr. The solvent was removed by evaporation and the residue was washed with 0.1 N HCl and H_2O, and reprecipitated from DMF and AcOEt; yield 1.06 g (68%), mp 259—261° (dec.), $[\alpha]_D^{23}$ −49.6° (c=1, AcOH). *Anal.* Calcd. for $C_{53}H_{74}N_8O_{11}S_2$: C, 59.9; H, 7.0; N, 10.6. Found: C, 59.6; H, 7.1; N, 10.5. Amino acid ratios in an acid hydrolysate; Leu 1.9; Gly 1.0; Glu 1.2; Ile 1.0; Cys was not determined (average recovery 98%). H–Leu–Gly–Cys(SO_3^-)–Gln–Ile–Cys(SO_3^-)–Leu–OH was derived from the partially protected heptapeptide (B) in the manner described previously. Twenty mg of B was reduced with sodium in liquid ammonia (50 ml) and sulfitolyzed with sodium tetrathionate (60 mg) and sodium sulfite (150 mg) in 8 M guanidine hydrochloride (2 ml). The S-sulfonated product was purified by gel-filtration on a Sephadex G-15 column (1.0×170 cm) in 5% AcOH. The effluent (tube Nos. 50—58, 2 ml each) was lyophilized and rechromatographed to give the purified product; yield 12 mg (70%), Rf^2 0.33 (ninhydrin stain). Amino acid ratios in an acid hydrolysate; Leu 1.8; Gly 1.0; Glu 1.1; Ile 1.0 (average recovery 75%) and amino acid ratios in an AMP digest; Leu 2.0; Gly 1.0; Gln 0.8; Ile 1.1; S-sulfoCys 1.9 (average recovery 70%).

Formation of the Iron-sulfur Complex——(a) To a solution of A (50 mg) in liquid ammonia (100 ml), small pieces of Na were added at the boiling point of ammonia until the blue color remained for 10 sec. A small amount of ammonium chloride was added to the blue-colored solution and ammonia was removed completely. The residue was dissolved in 6 ml of 10 mM Tris buffer (pH 7.5) containing mercaptoethanol (75 mg), and this solution was allowed to stand at room temperature for 30 min. To this solution, Na_2S ($Na_2S \cdot 9H_2O$, 85 mg) and $FeCl_3$ (38 mg) were added. This reaction mixture was kept at 0° for 2 hr and then centrifuged. The absorption spectrum of the brown-colored supernatant is shown in Fig. 4. (b) Formation of the iron-sulfur complex of deblocked B was carried out in the same way.

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