Table I. Selected Distances[a] (Å) and Angles (deg) in the $(Et_4N)^+$ Salts of the $[(Fe_6S_6X_6)(Mo(CO)_3)_2]^{n-}$ Ions (A, n = 4, X = Cl^-; B, n = 3, X = Cl^-; C, n = 3, X = Br^-)

	A[b]	B[c]	C[c]
	Distances		
Fe-Mo	3.005 (3, 11)	2.929 (2,2)	2.946 (3, 17)[d]
Fe-Fe[e]	3.785 (3, 10)	3.761 (2, 3)	3.755 (3, 6)
Fe-Fe[f]	2.761 (3, 10)	2.744 (2, 3)	2.733 (3, 10)
Mo-S	2.619 (3, 3)	2.579 (2, 3)	2.572 (3, 7)
Fe-S[e]	2.333 (3, 3)	2.314 (2, 4)	2.318 (3, 4)
Fe-S[f]	2.286 (6 5)	2.282 (4, 4)	2.277 (6, 5)
Fe-X	2.245 (3 3)	2.225 (2, 3)	2.355 (3, 4)
Mo-C	1.958 (3, 11)	2.01 (2, 2)	1.98 (3, 2)
	Angles		
S-Mo-S	93.4 (3, 2)	95.8 (2, 1)	95.2 (3, 7)
Fe-Mo-Fe	78.07 (3, 2)	79.9 (2, 1)	79.2 (3, 2)
Fe-S-Fe[e]	111.8 (3, 5)	111.0 (2, 3)	111.1 (3, 7)
Fe-S-Fe[f]	73.4 (6, 2)	73.3 (4, 3)	73.0 (6, 2)
S-Fe-S[e]	113.0 (3, 4)	114.1 (2, 3)	113.1 (3, 5)

[a] The mean values of chemically equivalent bonds are given. In parentheses the first entry represents the number of independent distances or angles averaged out and the second entry represents the larger of the standard deviations for an individual value estimated from the inverse matrix or of the standard deviation, $\sigma = [\sum_{i=1}^{N} (x_i - x)^2/N(N-1)]^{1/2}$. [b] From ref 8. [c] This work. [d] Range: 2.913 (2)-2.954 (2) Å. [e] Distances or angles within the Fe_3S_3 structural units. [f] Distances or angles within the Fe_2S_2 rhombic units.

vs. SCE) in CH_3CN solution, however, is inadequate for the reduction of the $[Fe_6S_6(OR)_6(Mo(CO)_3)_2]^{3-}$ adduct. The latter shows the potential for the 3-/4- couple at -0.35 V and is the only product isolated in the reaction of $[Fe_6S_6(OR)_6]^{3-}$ with excess $Mo(CO)_3(CH_3CN)_3$. The $[Fe_6S_6(Cl)_6(Mo(CO)_3)_2]^{3-}$ adduct, (I) can be obtained in ~60% yield by the reaction of $[Fe_6S_6(Cl)_6]^{3-}$ with $Mo(CO)_3(CH_3CN)_3$ in a 4:1 molar ratio, in dichloroethane[11] at 70-80 °C for ~50 min. The synthesis of I and the corresponding Br^- analogue II, also can be accomplished in similar yields by the reactions between the $[Fe_6S_6(X)_6(Mo(CO)_3)_2]^{4-}$ complexes and $[Fe(C_5H_5)_2][PF_6]$ in a 1:1 molar ratio in CH_3CN solution. The crystalline Et_4N^+ salts of I and II obtained in this fashion are X-ray isomorphous. A different crystalline modification of II is obtained when the oxidation is carried out with the oxidant in a 2:1 molar excess. The structure of this monoclinic modification is reported herein (vide infra).

The purity of I and II can be monitored conveniently by infrared spectroscopy. The C-O stretching vibrations in I and II occur as sharp doublets in the infrared spectra[12] at 1918, 1945 cm^{-1} and 1912, 1948 cm^{-1}, respectively, and reflect the C_{3v} microsymmetry of the coordinated $Mo(CO)_3$ units. These values are higher than those found in the corresponding tetraanions at 1847, 1908 cm^{-1} and 1852, 1912 cm^{-1} respectively for the chloro and bromo homologues and suggest that the oxidation of the tetraanions involves a molecular orbital with considerable Mo character. The electronic spectrum of I shows an absorption band at 460 nm (e = 11 500), while that of II shows bands at 470 (sh) and 314 nm (e = 22 150).

Single crystals of I and II were obtained by the slow diffusion of ether to CH_3CN solutions of these compounds. The crystal structures[13] of the trianions in both I and II are very similar and contain the $[Fe_6Mo_2S_6]^{3+}$ cores (Figure 1). The two crystallographically independent anions in the structure of I are situated on special positions ($\bar{3}$-site symmetry) and possess nearly exact D_{3d} symmetry. In the structure of II the anions are located on crystallographic centers of symmetry. Selected structural parameters for these anions are shown in Table I. As observed previously,[8] coordination of the $Mo(CO)_3$ to the Fe_6S_6 central cages results in an elongation of the latter along the $\bar{3}$-axis in I and the idealized $\bar{3}$-axis in II. A structural comparison between I and the corresponding tetraanion III (Table I) shows a significant shortening of the Mo-Fe and the Mo-S distances in the former and no significant differences within the Fe_6S_6 frameworks in the two structures. The data indicate that the highest occupied MO in III consists mainly of Mo and S atomic functions and is antibonding in character.

The isomer shifts (IS) and quadrupole splittings (Δ_{Eq}) in the ^{57}Fe Mössbauer spectra of I at 0.56 and 1.00 mm/s and of II at 0.59 and 1.06 mm/s, respectively (vs. Fe, T = 92 K), are quite similar to those of the corresponding tetraanions at 0.63, 1.00 mm/s and 0.62, 1.02 mm/s. These results, and the insignificant structural differences found in the Fe_6S_6 cores in both the 3- and 4- levels of the adducts, further reinforce the conclusion that the oxidation of the tetraanionic $Mo(CO)_3$ adducts is centered primarily on the Mo atoms. The isomer shifts in I, II, and their corresponding tetraanions are significantly larger by comparison to those in the "parent" $[Fe_6S_6(L)_6]^{3-}$ clusters (IS ~ 0.49 mm/s, Δ_{Eq} ~ 1.10 mm/s vs. Fe, T = 125 K). The data suggest that in the $[Fe_6S_6(L)_6(Mo(CO)_3)_2]^{n-}$ adducts the Fe_6S_6 cores assume an oxidation level lower than the one in the $[Fe_6S_6(L)_6]^{3-}$ clusters. In a formal sense both oxidation levels of the adducts can be described as containing the $[Fe_6S_6]^{2+}$ core. It appears that a reduction of the Fe_6S_6 core is important for the stability of either the 3- or the 4-levels of the adducts and may be able to stabilize adducts with Mo atoms in even higher formal oxidation states. The synthesis of the latter is an objective of future studies in our laboratory.

Acknowledgment. This research was supported by a grant from the National Institutes of Health (GM-26671).

Supplementary Material Available: Table of crystal and refinement data, positional and thermal parameters and intramolecular distances for I and II (6 pages); tables of structure factors for I and II (14 pages). Ordering information is given on any current masthead page.

(11) In CH_2Cl_2 solution the $Mo(CO)_3(CH_3CN)_3$ complex undergoes a one-electron irreversible oxidation on a Pt anode at 0.18 V vs. SCE.

(12) The infrared spectra reported were obtained in NaCl cells in CH_3CN solution.

(13) Crystal and refinement data for I and II can be found in the supplementary material.

Peptide Segment Coupling Catalyzed by the Semisynthetic Enzyme Thiolsubtilisin

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Received February 23, 1987

Stepwise solid-phase peptide synthesis[1] has developed in recent years to the stage where even the preparation of peptides greater than 100 amino acids in length has been undertaken.[2] Nevertheless, an alternative approach to the construction of very large peptides involving a combination of segment synthesis with segment condensation[3] remains very appealing. The segments that are the intermediates in this process can be purified and thoroughly characterized, reducing the need for laborious purification of the final product, which is often required for very large peptides produced by stepwise solid-phase synthesis. Further, the segment synthesis-condensation strategy is particularly adaptable to the preparation of analogues of naturally occurring peptides.

While the rapid synthesis of peptide segments of up to 10 amino acids is now feasible,[3,4] methods for the chemical couplng of such segments often suffer from problems such as racemization during

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(3) For example, a 44 amino acid peptide model of apolipoprotein A-I was recently prepared by this route. Nakagawa, S. H.; Lau, H. S. H.; Kezdy, F. J.; Kaiser, E. T. *J. Am. Chem. Soc.* **1985**, *107*, 7087.

(4) A *p*-nitrobenzophenone oxime derivative of polystyrene has been utilized in the facile preparation of peptide segments as described in: DeGrado, W. F.; Kaiser, E. T. *J. Org. Chem.* **1980**, *45*, 1295; **1982**, *47*, 3258.

0002-7863/87/1509-3808$01.50/0

Scheme I[a]

$$\text{P-AA}_1\text{-OC}_6\text{H}_4\text{Cl-}p + \underset{(\text{NH}_2)}{\text{H}_2\text{N-AA}_2\text{-OH}} \xrightarrow[\text{DMF-H}_2\text{O}]{\text{thiolsubtilisin}} \underset{(\text{NH}_2)}{\text{P-AA}_1\text{-AA}_2\text{-OH}} + \text{HOC}_6\text{H}_4\text{Cl-}p$$

[a] P = benzyloxycarbonyl (Z) or fluorenylmethoxycarbonyl (FMOC) group, AA_1 = N-terminal amino acid or peptide component, and AA_2 = C-terminal amino acid or peptide component.

the coupling of the segments, the low solubility of side-chain-protected peptides in the organic solvents employed, and low yields. For such reasons, protease-catalyzed peptide synthesis[5] has drawn considerable attention because most of the enzyme-catalyzed reactions are highly stereoselective, racemization-free, and required minimal side-chain protection[6] in the coupling steps. However, a limitation in the uses of proteases in peptide synthesis is that because of the attention that must be given to the problem of the position of equilibrium between the final peptide product and its constituent amino acids or peptide segments the reaction conditions (reaction time, pH of the medium, temperature, and solvent system) must be adjusted for each coupling.

We reasoned that an endopeptidase "damaged" in such a way that it acts as a poor catalyst for peptide hydrolyses, but which retains the ability to be acylated at its active site by a peptide segment active ester and then to be deacylated through attack by the N-terminal amino group of an another peptide segment, could have important applications in the development of a general methodology for peptide segment condensation. We wish now to report that the semisynthetic enzyme thiolsubtilisin, first prepared independently by Bender[7] and Koshland[8] and their coworkers, which possesses these properties can act as an effective catalyst in the synthesis of peptides by the route outlined in Scheme I.

Thiolsubtilisin is well characterized[9] and easily prepared[10] in two steps from the inexpensive, commercially available protease subtilisin. Since thiolsubtilisin is a very poor protease, the peptide bonds formed in the process of Scheme I are not significantly cleaved during the course of reaction. To date, the active ester derivatives we have found to be most suitable for coupling catalyzed by this semisynthetic enzyme are *p*-chlorophenyl esters.[11] In the initial phases of our work we explored the specificity requirements for amino acids as acyl donors and acyl acceptors in the thiolsubtilisin-catalyzed preparation of small peptides. The results are shown in Table I. Several interesting features of the reaction were elucidated: (a) the L and D isomers of the Phe employed as the acyl component were strictly distinguished by thiolsubtilisin and only the L isomer was reactive; (b) protection of the hydroxyls of Ser and Tyr and the C-terminal carboxyl group of the acyl acceptor was unnecessary; (c) the substrate specificity of thiolsubtilisin was quite broad.

These favorable results in the synthesis of small peptides encouraged us to apply thiolsubtilisin in the preparation of higher molecular weight peptides such as [Leu][5] enkephalin amide (eq 1) and the fragment of ribonuclease T_1 corresponding to residues 12–23 (RNase T_1, 12–23),[12] which contain five and 12 amino acid residues, respectively.

Table I. Coupling Yields in the Synthesis of Small Peptides Catalyzed by Thiolsubtilisin[a] (See Scheme I)

acyl donor	acyl acceptor	yield,[b] %
Z-L-Phe	Gly NH_2	quant. (95)[c]
Z-D-Phe	Gly NH_2	0[d]
Z-L-Ser	Gly NH_2	91
Z-L-Tyr	Gly NH_2	90
Z-L-Val	Gly NH_2	0[e]
Z-L-Phe	Val NH_2	90
Z-L-Phe	Tyr NH_2	90 (65)[c]
Z-L-Phe	Pro NH_2	0[e]
Z-L-Phe	Ser-Gly	quant. (90)[c]
Z-L-Phe	Gly-Phe NH_2	80

[a] Experimental conditions unless otherwise stated: acyl donor, 1 mM; acyl acceptor, 20 mM; thiolsubtilisin, 14 μM; $PhB(OH)_2$, 0.1 μM; DMF/0.1 M phosphate buffer = 2/3, v/v; pH 8.0; room temperature 10 min–2 h. When *p*-chloromercuriobenzoate was added no coupling was observed over a period of several hours. The reaction course was monitored by HPLC (ODS reverse-phase column, acetonitrile/H_2O/TFA (0.1%)). The NMR spectra (360 MHz) for all products were in good agreement with their proposed structures. In nearly all cases the products were compared to authentic samples by HPLC. [b] Yields were estimated from HPLC analysis and are based on acyl donor. [c] Isolated yield is given in parentheses; acyl donor, 8.4 mM; acyl acceptor, 11.0 mM; thiolsubtilisin, 18 μM. [d] No reaction occurred in 2 h. [e] *p*-Chlorophenyl ester hydrolyzed to give Z-amino acid.

$$\underset{32\text{ mM (17.4 mg/800 }\mu\text{L})}{\text{Z-Tyr-Gly-Gly-Phe-OC}_6\text{H}_4\text{Cl-}p} + \underset{50\text{ mM}}{\text{LeuNH}_2} \xrightarrow[\text{pH 8.0, 0.1 M phosphate buffer/DMF (50/50, v/v), room temperature, 5 h}]{\text{thiolsubtilisin (19 }\mu\text{M)}} \underset{67\%\text{ yield of pure product}}{\text{Z-Tyr-Gly-Gly-Phe-Leu-NH}_2} + \text{HOC}_6\text{H}_4\text{Cl-}p \quad (1)$$

The procedure for the preparation of RNase T_1 (12–23) was as follows. To a DMF solution (2.6 mL) of FMOC-Ser-Ser-Ser-Asp-Val-Ser-Thr-Ala-OC_6H_4Cl-*p* (39 mg, 36 μmol) and $PhB(OH)_2$ (10 mg, 82 μmol)[14] were added at room temperature a solution of Gln-Ala-Ala-Gly (15 mg, 43 μmol) in 0.1 M phosphate buffer (1.59 mL, pH 8.3) and then a solution of 0.7×10^{-4} M thiolsubtilisin (0.59 mL, 0.01 M phosphate buffer, pH 7.0, containing 1 mM EDTA and 0.07 M KCl). After the mixture was stirred for 30 min, 100 μL of trifluoroacetic acid was added to stop the reaction, DMF was added, and the insoluble materials were removed by filtration. The product was purified by high-performance liquid chromatography on an Altex ODS reverse-phase column. The pure product (22 mg, 47% yield) was isolated as a single peak on elution with acetonitrile:H_2O:trifluoroacetic acid = 70:30:0.1, v/v/v. For [Leu][5]enkephalin amide and RNase T_1 12–23 (from which the FMOC group had been removed by piperidine/DMF, 60/40, v/v) the amino acid analyses obtained after hydrolysis with 6 N HCl at 110 °C for 22 h were the following: Gly(2) 1.94; Leu(1) 1.10; Phe(1) 1.04; Tyr(1) 0.97 and Ala(3) 2.98; Asx(1) 1.0; Glx(1) 1.08; Gly(1) 1.02; Ser(4) 3.65; Thr(1) 0.87; Val(1) 0.98, respectively.

The utility of thiolsubtilisin as a catalyst for the synthesis of peptides by segment condensation encourages us to apply this semisynthetic enzyme to the synthesis of large peptides. We are also pursuing the preparation and characterization of other modified endopeptidases with different specificities which will enlarge the repertoire of enzymes that can be employed in the

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(13) Detailed procedures for the preparation of the starting peptide segments used will be described in a future publication.

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construction of a variety of peptides.

Acknowledgment. The partial support of this research by National Science Foundation Grant CHE-8218637 is gratefully acknowledged.

DNA Binding Specificity of the Gold(III) Complex $(C_2H_5)_3PAuBr_3$

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Received January 20, 1987

Considerable attention has recently been focused on the antitumor agent *cis*-diaminedichloroplatinum(II) (cisplatin, **1**), which binds to purine sites located within the major groove of DNA. The origin for the antitumor effects of the compound lies in its ability to form an intrastrand cross-link between bases located on the same strand of DNA.[1] Compounds of gold have also been shown to have antitumor effects.[2] Studies attempting to uncover the chemical and biochemical events which may underlie the cytotoxicities of these agents have found that Au(I) and Au(III) complexes can bind to DNA in vitro and apparently can cleave DNA in cell culture.[3] In light of the close chemical similarity between the compounds of platinum and gold and the possibility that both may use DNA as a biological target site, we studied the binding of the square-planar Au(III) complex $(C_2H_5)_3PAuBr_3$ (**2**) to a *Hind III/NciI*, 139 base pair restriction fragment of pBR-322 DNA using DNA sequencing methodology.[4,5]

The sites of gold binding on the 3′-end-labeled restriction fragment[4] were uncovered by treating the metalated DNA with either dimethyl sulfate (DMS) or formic acid under mild conditions (37 °C for 10 min), followed by heating in the presence of excess piperidine to produce a strand break in the polymer.[6,7] The sites of strand scission in the absence and presence of various amounts of **2** were in turn determined via separation of the resulting oligonucleotide products by using high-resolution polyacrylamide gel electrophoresis and scanning of the resulting autoradiogram with microdensitometry.[5]

Densitometric scans of autoradiograms resulting from cleavage of the gold-bound restriction fragment with DMS/piperidine and HCO_2H/piperidine are shown in Figures 1 and 2. As is evident from the difference scan shown in Figure 1, the concentrations of oligomers resulting from DMS/piperidine treatment of the fragment decrease in the presence of **2**. This effect appears to be independent of sequence (all guanines are affected) and to increase with increasing concentration of **2**. Since the reaction of DMS with DNA results in methylation of the N-7 position of guanine, the most likely explanation for the observed gold-induced inhibition is that **2** is bound directly to N-7 of the heterocycle and is blocking alkylation of this site. A similar blocking effect has been previously observed for $[Pt(dien)]^{2+}$, where dien is diethylenetriamine, bound to the N-7 position of the nucleoside, guanosine.[8] This conclusion, binding of **2** to N-7 of guanine, was confirmed by examining the acid-catalyzed depurination of the fragment in the presence of the gold compound. As is evident from the difference scan shown in Figure 2, the gold complex

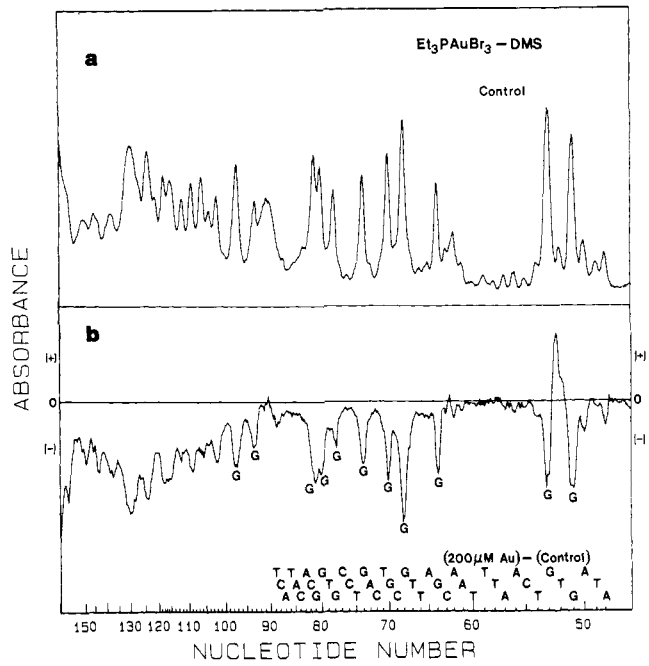

Figure 1. Microdensitometric scans of autoradiographic data involving dimethyl sulfate/piperidine treatment of the restriction fragment in the presence and absence of **2** are shown. Absorbance scan of the control (**a**), no **2**, and difference scan of 200 μM **2** – control (b). The small amount of background cleavage at all sites of the polymer, determined from controls, was due to DNA cleavage in the presence of only hot piperidine (Ambrose, B. J. B.; Pless, P. C. *Biochemistry* **1985**, *24*, 6194). The broad peak at position ~90 and the positive peak in the difference scan at position 52 were due to defects on the autoradiogram.

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(7) Metalation of the restriction fragment with **2** was carried out in a 50 mM Tris-Cl, pH 7.5 (0.1 mM EDTA) buffer in a total volume of 6 μL containing ~3 μM base pairs of the restriction fragment and **2** at four different concentrations in the range (2×10^{-4})–(2×10^{-7}) M. The stock solutions of **2** were freshly prepared in a 1:1 DMF/buffer solution. After incubation for 3 h at 37 °C, the gold-DNA solutions were treated with either 2 μL of 0.2% DMS in a 1:1 DMF/H_2O solution or 2 μL of a 20% aqueous solution of HCO_2H for 10 min at 37 °C. The reactions were terminated by addition of 12 μL of 20% aqueous piperidine and heating to 90 °C for 30 min. The resulting mixtures were frozen (−78 °C), lyophilized to dryness, and washed twice with 10 μL of methanol and the residue was taken up in 20 μL of the electrophoresis loading buffer,[5a] containing 100 mM mercaptoethanol. The 1:1 correspondence between the cleavage products and those produced by DNase I digests of the fragment indicated that the mercaptoethanol removed **2** from DNA. Control reactions in the absence of **2** confirmed that the aforementioned conditions resulted in ~80% uncleaved DNA, indicating that the products were statistically the result of a single cleavage on the full-length fragment. Electrophoresis, autoradiography, microdensitometry, and establishment of sequence were as earlier described.[4,5] The observed changes in oligonucleotide concentration were significant and well outside the limits of error of the measurement.[5b,c]

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