duction process because of the possible interference of oxygen. Their kinetics of photoreduction also appear to be different.

The procedure(s) reported here for the selective separation of gold, platinum, and rhodium is relatively simple and can be extended to the separation of other metals (e.g., silver(I) from palladium(II)). Another parameter that we are investigating[7] is the use of complexing agents, specific for a metal, that might lead to a more efficient or alternative route(s) in the reductive separation of metals on TiO_2.

Summary

We have described a general method for the selective separation of precious metals that is based on commercially available inexpensive materials such as TiO_2,[4] and on AM1 simulated sunlight. We have shown that pH, oxygen, and TiO_2 concentration can vary the rate of deposition of gold, platinum, palladium, and rhodium on TiO_2 and can lead to a differently coated TiO_2 powder. The present results also suggest the possibility of loading semiconductor powders (or colloids) with varying amounts of the noble metals that could find use in photocatalysis,[2c] a direction where much remains to be done to understand the mechanisms and the real potentials of photochemistry to practical and useful applications.

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Registry No. $PdCl_2$, 7647-10-1; $RhCl_3$, 10049-07-7; CH_3OH, 67-56-1; O_2, 7782-44-7; $AuCl_3$, 13453-07-1; H_2PtCl_6, 16941-12-1; Pd, 7440-05-3; Rh, 7440-16-6; Au, 7440-57-5; Pt, 7440-06-4; TiO_2, 13463-67-7.

Contribution from Abbott Laboratories, North Chicago, Illinois 60064, The W. R. Kenan Laboratories of Chemistry, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514, and Departments of Biochemistry and Surgery, Case Western Reserve University School of Medicine, Cleveland Metropolitan General Hospital, Cleveland, Ohio 44109

Terbium Ion Binding to a Synthetic γ-Carboxyglutamic Acid Containing Heptapeptide Corresponding to Bovine Prothrombin Residues 17-23

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Interactions of terbium(III) with a synthetic γ-carboxyglutamic acid- (GLA-) containing loop corresponding to bovine prothrombin residues 17-23 (^{17}Gla-Cys-Leu-Gla-Gla-Pro-^{23}Cys) are reported. The results are compared with metal ion binding properties of intact bovine prothrombin fragment 1, bovine prothrombin residues 1-39, and simple Gla- and Gla-Gla-containing peptides. The heptapeptide (Loop) forms a 2:1 metal:peptide complex with dissociation constants of 5.2 and 2.4 μM for the PM and PM_2 complexes. The two Loop-bound terbium(III) ions have each lost approximately 5.6 water molecules from their inner coordination spheres. Addition of excess lanthanum(III) to terbium(III)-containing Loop displaces only a portion of the terbium(III). Analysis of the decay of luminescence of the terbium(III):Loop system in the presence of lanthanum(III) reveals the existence of multiexponential decay, suggesting that the system is no longer in fast exchange on the time scale of the terbium(III) excited-state lifetime. Both the extent of dehydration of Loop-bound terbium(III) ions and the effect of competing ions on exchange between Loop-bound and free aquo ion are more characteristic of the intact protein than of simple Gla-containing peptides. We conclude that the 17-23 structure itself plays a central role in determining the functional metal ion binding properties of the intact prothrombin molecule.

Introduction

Characterization of the interaction of metal ions with the blood-clotting proteins is a matter of considerable importance since it is the metal ion stabilized forms of these proteins that interact with phospholipid-containing cell surfaces and catalyze many of the processes leading to formation of the hemostatic clot.[1] Prothrombin, the zymogen form of the enzyme thrombin, is an excellent model for the study of metal ion interactions with many of these proteins. Near the amino terminus of this protein is a region containing an unusual amino acid, γ-carboxyglutamic acid (Gla). This amino acid is formed by a post-translational vitamin K-dependent carboxylation of glutamyl residues.[2] A considerable number of studies have implicated the Gla residues in the phospholipid binding process, which is essential for function of these proteins in normal blood coagulation.[2]

The Gla-containing region of bovine prothrombin involves at least the first 33 amino terminal residues. The 10 glutamyl residues in this region are all present as Gla. The Gla residues appear three times in this sequence in pairs. The conformational response of the following portion of the bovine sequence (Chart I) has been investigated intensively by experimental and computational methods since isomerization about the ^{21}Gla-^{22}Pro peptide bond has kinetic consequences for protein folding when metal ions are added to the metal ion free protein.[3-6] Since a particular configuration of this loop region is required before appropriate folding in the remainder of the Gla region can occur, leading ultimately to phospholipid binding, it appears that metal ion binding in this region may play an important part in stabilization of the functional structure of prothrombin.

Chart I

^{17}Gla-^{18}Cys-^{19}Leu-^{20}Gla-^{21}Gla-^{22}Pro-^{23}Cys

Although calcium and magnesium ion binding are of primary importance in in vivo processes involved in blood coagulation,[7]

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(1) Nelsestuen, G. L. *Met. Ions Biol. Syst.* **1984**, *17*, 353-381.
(2) Suttie, J. W. *Ann. Rev. Biochem.* **1985**, *54*, 459-477.
(3) Nelsestuen, G. L. *J. Biol. Chem.* **1976**, *251*, 5648-5656.
(4) Marsh, H. C.; Scott, M. E.; Hiskey, R. G.; Koehler, K. A. *Biochem. J.* **1979**, *183*, 513-517.
(5) Eastman, M. A.; Pedersen, L. G.; Hiskey, R. G.; Pique, M.; Koehler, K. A.; Gottschalk, K. E.; Nemethy, G.; Scheraga, H. A. *Int. J. Pept. Protein Res.* **1986**, *27*, 530-553.
(6) Madar, D. A.; Hall, T. J.; Reisner, H. M.; Hiskey, R. G.; Koehler, K. A. *J. Biol. Chem.* **1980**, *255*, 8599-8605.
(7) Prendergast, F. G.; Mann, K. G. *J. Biol. Chem.* **1977**, *252*, 840-850.

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we and others have utilized the lanthanide ions as a means of probing the Gla-containing region.[8-10] We report here an investigation of the interaction of terbium ions with a synthetic heptapeptide corresponding to the structure in Chart I. These results are compared with those previously obtained for europium ion binding to simple Gla and Gla-Gla structures.[8,11]

Experimental Section

Chemicals. $TbCl_3 \cdot 6H_2O$ was obtained from Aldrich Chemical Co. All water used was distilled, deionized, and organic free (Barnstead Nanopure System). The cyclic heptapeptide Z-L-Gla-L-Cys-L-Leu-L-Gla-L-Gla-L-Pro-L-Cys-Obzl was synthesized and purified as described elsewhere.[12]

Fluorescence Spectroscopy. Fluorescence measurements were made on a Perkin-Elmer LS-5 spectrofluorometer with pulsed light source, operating in its phosphorescence mode. The "delay" (T_d) is the time between the lamp flash and the beginning of data acquisition. The "gate" (T_g) is the duration of data acquisition. A 410-nm cutoff filter was inserted in the emission path. Samples were dissolved in 5 or 20 mM HEPES, 0.1 M NaCl, pH 7.0, and runs were thermostated at 25 °C. Terbium(III) binding was followed by either the increase in the terbium(III) excited-state lifetime or by the increase in terbium(III) luminescence emission at 545 nm after direct terbium excitation at 230 nm. Appropriate inner filter-effect corrections were applied.[13] Optical densities were obtained on either Perkin-Elmer Lambda-5 or Beckman DU7 spectrophotometers. Front surface techniques were employed for studies carried out under conditions favoring stoichiometric binding of Tb^{3+} and heptapeptide loop ([heptapeptide] = 20×10^{-5} M and $[Tb^{3+}]$ varied up to 44×10^{-5} M) because of sample turbidity.

Models. The data from the peptide titration experiments were compared with theoretical curves calculated by assuming three thermodynamic equilibrium models in order to assess the probable ion:loop stoichiometry and the magnitude of the equilibrium dissociation constants involved.

model 1

$$P + M \underset{K_1}{\rightleftharpoons} PM \tag{1}$$

where $K_1 = [P_f][M_f]/[PM]$; $[P_f]$ and $[M_f]$ are the free peptide and metal ion concentrations, respectively. Substitution of $[P_f] = [P_t] - [PM]$ and $[M_f] = [M_t] - [PM]$ (where $[P_t]$ and $[M_t]$ refer to total concentrations) leads to the following quadratic equation:

$$0 = [PM]^2 - ([M_t] + [P_t] + K_1)[PM] + [P_t][M_t] \tag{2}$$

This equation is solved for [PM]. The quantities $[P_f]$ and $[M_f]$ may then be determined.

model 2

$$P + M \underset{K_1}{\rightleftharpoons} PM \qquad PM + M \underset{K_2}{\rightleftharpoons} PM_2 \tag{3}$$

where $K_1 = [P_f][M_f]/[PM]$ and $K_2 = [M_f][PM]/[PM_2]$ yields the following cubic equation:

$$f(M_f) = 0 = [M_f]^3 + (K_2 + 2[P_t] - [M_t])[M_f]^2 + (K_1 + [P_t] - [M_t])K_2[M_f] - K_1K_2[M_t] \tag{4}$$

Reuben[14] has utilized an iterative approach based on Newton's method to solve this equation. The cubic equation can be solved by successive iterations of

$$[M_f]^{n+1} = [M_f]^n - [f(M_f)^n/f'(M_f)^n] \tag{5}$$

where $f'(M_f)$ represents the first derivative of $f(M_f)$.

model 3

$$P + M \underset{K_1}{\rightleftharpoons} PM \qquad PM + P \underset{K_2}{\rightleftharpoons} P_2M \tag{6}$$

This model involves two peptides bound to a single terbium ion. This set of equilibria becomes identical with the second model when all P and M labels are exchanged. Hence, the associated equations can be derived by inspection.

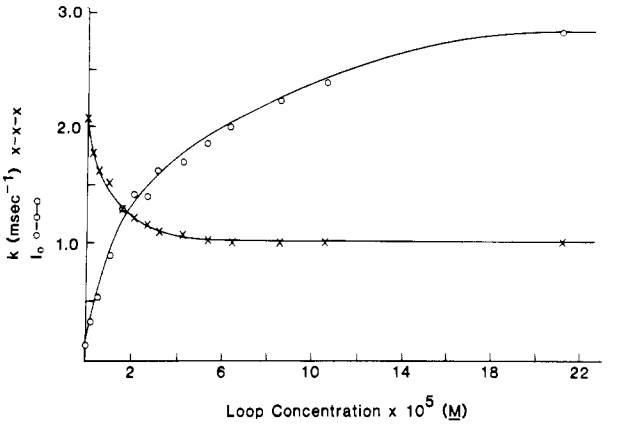


Figure 1. Titration of terbium(III) chloride with the synthetic heptapeptide corresponding to residues 17–23 of the bovine prothrombin sequence. The terbium ion concentration was 10.1 μM. The buffer employed was 0.010 M Hepes, 0.1 M NaCl, pH 7.0. Heptapeptide stock solution was 5.33×10^{-4} M in buffer. Excitation was at 230 nm with a 15-nm slit. Emission was at 545 nm with a 20-nm slit. $T_g = 0.020$ ms. T_d was varied. A 410-nm cutoff filter was utilized on the emission side. I_0 values are corrected for inner filter effects.

The theoretical curves are compared to the observed curve by means of the rms deviation function, which is minimized over the entire grid of variables. The rms deviation is given by the formula

$$\text{rms error} = \sum_i (ob_i - th_i)^2/(n_N - p_N) \tag{7}$$

where ob_i = observed experimental value, th_i = theoretically calculated value, n_N = number of experimental data points, and p_N = number of theoretical variables. Since the rms error value is normalized with respect to the number of variables, results from different models can be compared with one another. For model 2, th_i is

$$th_i = \tau_{M_f}\frac{[M_f]}{[M_t]} + \tau_{PM}\frac{[PM]}{[M_t]} + 2\tau_{PM_2}\frac{[PM_2]}{[M_t]} \tag{8}$$

Results

The strong terbium(III) excitation band at 215 nm was employed for direct excitation of terbium. Emission was monitored at 545 nm. Terbium ion luminescence decay in this aqueous system was exponential. The excited-state lifetime (the reciprocal of the exponential decay rate constant), τ^{-1}_{obsd}, is known to be composed of several terms:

$$\tau^{-1}_{obsd} = \tau^{-1}_{nat} + \tau^{-1}_{nonrad} + \tau^{-1}_{OH} \tag{9}$$

For terbium(III) in aqueous solution, the intrinsic rate for the emission of photons (τ^{-1}_{nat}) is 0.11 ms^{-1}, nonradiative deexcitation, which does not involve OH vibrations of coordinated water (τ^{-1}_{nonrad}) is 0.19 ms^{-1}, and nonradiative deexcitation channeled through the vibrational manifold of OH oscillators in the inner coordination sphere (τ^{-1}_{OH}) is 2.15 ms^{-1}. As water molecules are displaced from the inner coordination sphere of terbium by other ligands, the τ^{-1}_{OH} term decreases linearly and vanishes in the absence of coordinated water. Thus, the τ^{-1}_{OH} term decreases the same amount with the loss of each water. As pointed out by Horrocks and his colleagues,[15,16] this allows the calculation of the number of water molecules displaced from the inner coordination sphere by chelating ligands. In the absence of heptapeptide, the observed terbium ion luminescence decay rate was 2.30 ms^{-1}, very close to the sum calculated by using eq 9. An extrapolation to 100% D_2O (i.e. no OH oscillators) yielded a decay rate constant of 0.20 ms^{-1} in our system. Since terbium(III) is believed to coordinate nine water molecules in aqueous solution, the observed rate differences between terbium ion in water and in D_2O rep-

(8) Sarasua, M. M.; Scott, M. E.; Helpern, J. A.; Ten Kortenaar, P. B. W.; Boggs, N. T., III; Pedersen, L. G.; Koehler, K. A.; Hiskey, R. G. *J. Am. Chem. Soc.* **1980**, *102*, 3404–3412.
(9) Horrocks, W. D., Jr.; Albin, M. *Prog. Inorg. Chem.* **1984**, *31*, 1–104.
(10) Sommerville, L. E.; Thomas, D. D.; Nelsestuen, G. L. *J. Biol. Chem.* **1985**, *260*, 10444–10452.
(11) Marsh, H. C.; Sarasua, M. M.; Madar, D. A.; Hiskey, R. G.; Koehler, K. A. *J. Biol. Chem.* **1981**, *256*, 7863–7870.
(12) Hoke, R. A.; Deerfield, D. W.; Pedersen, L. G.; Koehler, K. A.; Hiskey, R. G. *Int. J. Pept. Protein Res.*, in press.
(13) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum: New York, 1983.
(14) Reuben, J. *J. Am. Chem. Soc.* **1973**, *95*, 3534–3540.
(15) Horrocks, W. D., Jr.; Sudnick, D. R. *J. Am. Chem. Soc.* **1979**, *101*, 334–340.
(16) Horrocks, W. D., Jr.; Schmidt, G. F.; Sudnick, D. R.; Kittrell, C.; Bernheim, R. A. *J. Am. Chem. Soc.* **1977**, *99*, 2376–2378.

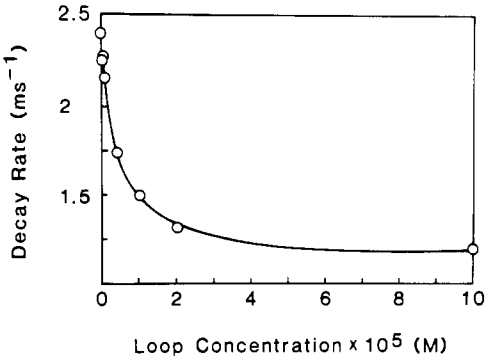


Figure 2. Fit of the PM_2 model to data obtained from a titration of 10.1 μM terbium(III) chloride with heptapeptide. Heptapeptide concentration was varied up to 100 μM. For further details, see Experimental Section. Line drawn through experimental points is calculated by assuming the PM_2 model, K_1 = 5 μM, K_2 = 2 μM, and that metal ion luminescence lifetimes in PM and PM_2 complexes are 1.1 ms^{-1}. Table I describes statistics of this and other attempted fits.

Table I. Fit of Stoichiometric Models to Experimental Terbium(III):Heptapeptide Lifetime Data

model[a]	K_1,[b] μM	K_2, μM	K_3, μM	rms[c]
PM	0.84			0.006 92
PM_2	5.24	2.39		0.000 87
P_2M	0.50		3.5	0.009 9

[a] Models are discussed in the Experimental Section. [b] K's (dissociation constants) are defined in the Experimental Section. [c] Goodness of fit is discussed in the Experimental Section.

resents nine water molecules displaced from the terbium ion inner coordination sphere. Thus, the decay rate change for each water displaced is 0.23 ms^{-1}. This conclusion compares favorably with the exhaustive study of Horrocks and Sudnick,[15] which yielded a decay rate change of 0.24 ms^{-1}/water molecule displaced.

Excitation of a 10.1 μM terbium(III) solution at 230 nm, monitoring emission at 545 nm, and varying the cyclic heptapeptide concentration from 0 to 213 μM yields the parabolic titration curve reproduced in Figure 1. The addition of heptapeptide lowers the τ^{-1}_{obsd} value by displacing water molecules from the bound terbium ion. Concomittantly, the observed terbium(III) emission intensity, I_0, increases with loop concentration. The limiting value of the decay rate at excess heptapeptide was 1.0 ms^{-1}. The observed limiting difference between aquoterbium(III) and terbium(III) in the presence of peptide corresponds to a displacement of approximately 5.6 water molecules from the inner sphere of the ion. Sommerville et al.[10] have calculated terbium(III) decay rates in the presence of bovine prothrombin fragment 1 species containing one and two bound terbium(III) ions, yielding terbium(III) decay rate constants of 0.79 and 0.81 ms^{-1} for the one and two terbium(III)-containing species, respectively. Such rate constants suggest that when bound to bovine prothrombin fragment 1, terbium(III) ions have lost as many as seven water molecules from their inner coordination sphere.

A second titration of terbium(III) with the cyclic heptapeptide was carried out and fits of the experimental results with three models involving formation of PM, PM_2, and P_2M complexes were attempted. For these studies a 10.1 μM terbium(III) solution was excited at 215 nm and emission was monitored at 545 nm. Heptapeptide concentration was varied up to 100 μM (Figure 2). Table I contains the results of attempted fits to the three equilibrium models discussed in the experimental section. The PM_2 model, which involves a complex containing one peptide and two metal ions, yields a significantly better fit to the experimental data than the PM or P_2M models. The line drawn in Figure 2 is calculated by assuming the PM_2 model, metal ion luminescence lifetimes of 1.1 ms^{-1} for terbium in both the PM and PM_2 complexes, and values of 5.24 and 2.39 μM for K_1 and K_2, respectively.

$$P + M \underset{5\ \mu M}{\overset{K_1}{\rightleftharpoons}} PM \underset{2\ \mu M}{\overset{K_2}{\rightleftharpoons}} PM_2 \qquad (10)$$

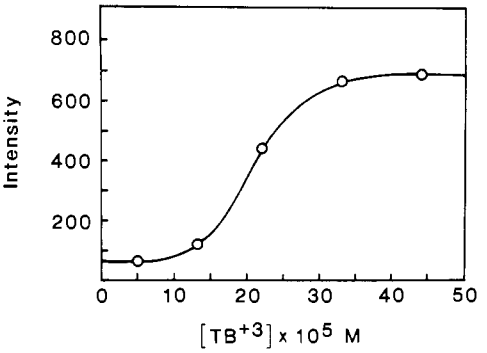


Figure 3. Stoichiometric titration of heptapeptide with terbium(III) chloride. Heptapeptide concentration was 20 × 10^{-5} M. Buffer employed was 0.010 M Hepes, 0.1 M NaCl, pH 7.0. Front surface detection was employed.

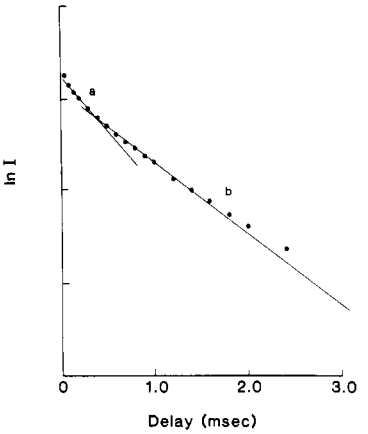


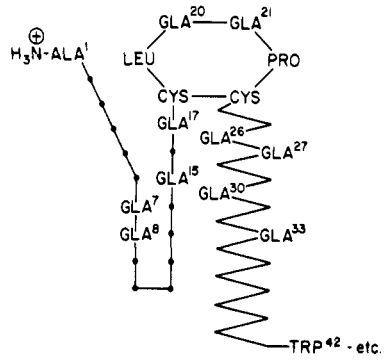
Figure 4. Semilogarithmic plot of the decay of terbium(III) emission in a sample containing 10.1 × 10^{-6} M terbium chloride, 10.7 × 10^{-5} M heptapeptide, and 9.1 × 10^{-4} M lanthanum chloride. Region a yields a lifetime of 1.12 ms^{-1} (R = 0.998); region b yields a lifetime of 0.77 ms^{-1} (R = 0.999).

As a result of either simple charge neutralization or exposure of hydrophobic regions, metal ion binding to Gla-containing peptides isolated from the amino terminal region of prothrombin leads to sample turbidity. However, the work of Horrocks and Sudnick[15] indicates that lanthanide ion nonradiative deexcitation via coordinated water is independent of the state of matter. Further evidence for the stoichiometry of the terbium:heptapeptide complex derives from a stoichiometric titration of heptapeptide with terbium(III) (Figure 3). Excitation was at 230 nm and emission was determined by utilizing front surface techniques at 545 nm because sample turbidity was high at the concentrations of heptapeptide and metal ion employed. No attempt was made to fit these results to specific models. The line drawn in Figure 3 is a visual fit. Fluorescence intensity increases in a sigmoidal fashion and levels off at a 2:1 terbium:heptapeptide mole ratio.

Work of Marsh et al.[11] and Sommerville et al.[10] indicates that, under certain circumstances, it is possible to slow the rate of exchange of metal ion into at least one metal ion binding site in bovine prothrombin fragment 1. For example, Marsh et al.[11] observed that addition of calcium or lanthanum ions to a europium(III):fragment 1 complex (1:1 or 2:1) leads to a "tightening of coordination" around the bound europium(III) ion(s). The following studies were carried out in order to determine, in a phenomenological sense, whether such processes occur in the interactions of the synthetic heptapeptide with lanthanide ions.

To a sample containing terbium(III) and heptapeptide (1.7 × 10^{-4} M heptapeptide, 1.0 × 10^{-5} M Tb^{3+}; k = 1.02 ms^{-1}), a 100-fold excess of lanthanum(III) (0.2 mL of 1 × 10^{-2} M lanthanum chloride added to 2.0 mL of the peptide:terbium sample) was added. The steady-state terbium ion luminescence intensity immediately decreased by 39% of the initial intensity. A further very slow decrease (less than 4%/h) was observed. Terbium ion

Chart II



emission decay in the lanthanum ion containing fragment 1:terbium ion sample was *not* a single exponential (Figure 4). A semilog plot of the decay of terbium(III) emission intensity yields observed rate constants at short and long delay times of approximately 1.1 and 0.8 ms^{-1}. Since the presence of more than two exponentials appears probable, component stripping was not attempted.

Discussion

As many as eight water molecules are displaced from the inner coordination sphere of those europium(III) ions that bind most tightly to bovine prothrombin fragment 1. Displacement of water molecules from the metal ion inner coordination sphere by Gla-containing small peptides is much weaker. For example, the peptide Z-Gly-D,L-Gla-Gly-OEt forms a 1:2 M:P complex with displacement of an average of four waters of hydration from the metal ion and dissociation constants of 5.9 and 1.5 μM for the PM and P_2M complexes, respectively. Z-D-Gla-D-Gla-OMe forms a 2:1 M:P complex with displacement of four water molecules from the europium(III) ions and values of 0.6 and 1.1 μM for K_1 and K_2.[8] The presumably more sterically hindered structure, Phe-L-Leu-L-Gla-L-Gla-L-Leu-OMe, forms instead a 1:1 M:P complex by displacing four waters from europium(III). The dissociation constant for the MP complex involving this pentapeptide is 4 μM. In none of these simple cases does water displacement approach the eight waters displaced from europium(III) by bovine prothrombin fragment 1.[11,16] Since the loop structure and the first approximately 42 amino terminal residues of bovine prothrombin, are likely to be involved in at least one, and perhaps two, metal ion binding sites crucial to the functional behavior of prothrombin, we have synthesized the three Gla-containing loop structure in order to determine whether the heptapeptide is intrinsically able to reflect the behavior of the intact protein.

The fundamental metal ion binding properties of the heptapeptide, 2:1 M:P complex formation with K_1 and K_2 equal to 5.2 and 2.4 μM, respectively, and displacement of 5.6 water molecules suggest greater similarities to the intact protein than to the Gla-Gla peptide systems. Fragment 1 appears to provide approximately one more coordinating ligand to bound terbium(III) than does the heptapeptide structure. The studies of Sommerville et al.[10] indicate that fragment 1 binds at least three terbium(III) ions tightly, in contrast to the two ions bound tightly by the loop. These observations suggest the potential importance of extraloop interactions to metal ion binding processes in the intact protein.

Molecular mechanics calculations,[5] observations based on simple model building, and conclusions based upon kinetic studies[4] suggest the following hypothetical sequence of events in bovine prothrombin metal ion binding. In the absence of metal ions, the protein exists with an equilibrium distribution of loop (^{18}Cys–^{23}Cys) trans and cis ^{21}Gla-Pro configurations (75%:25% trans:cis). In the trans configuration, loop Gla residues 20 and 21 are moved away from Gla-17. However, in the *cis*-Gla-Pro configuration, Gla residues 20 and 21 are situated above the plane of the loop in relatively close proximity to Gla-17. Perhaps most importantly, in the trans loop structure, the incoming 1–17 polypeptide chain and the outgoing 23–etc. chain are divergent, while in the cis conformation they are nearly parallel. Thus, not only site affinities themselves, but the observed cooperativity of tight metal ion binding to fragment 1 may be determined by interactions involving the loop and extraloop GLA residues. Considered in this light, the metal ion dependent trans → cis loop configuration change may be driven by metal ion stabilization of the approximation of the two polypeptide chains. The potential structures involved are illustrated in Chart II.

While the number of water molecules displaced from heptapeptide-bound metal ions suggests the number of involved ligands, no information is provided concerning their arrangement in space. The results of our previous Gla-Gla peptide europium(III) studies and those reported here for the heptapeptide loop may be compared. The two bound metal ions in the Gla-Gla dipeptide each lose approximately four waters while those in the heptapeptide loop 2:1 M:P complex lose 5.6 waters. The additional water displaced from Tb(III) in the heptapeptide:metal ion complex may either be the result of further steric constraints on access by water molecules to the complexed metal ion imposed by the presence of the plane of the ^{18}Cys–^{23}Cys loop or may indicate involvement of residues such as Gla residue 17 or Ser residue 24.

At appropriate concentrations of a competing ion, such as calcium or lanthanum, it is possible to establish conditions where fast exchange between pools of fragment 1-bound and free europium(III) or terbium(III) appears not to occur.[10,11] It is intriguing that the heptapeptide structure shows similar behavior in the presence of terbium(III) and lanthanum(III). Such behavior is not observed for simple Gla-containing peptides, such as Z-Gla-Ser-OMe, Z-Gla-Gla-OMe, or H-Phe-Leu-Gla-Gla-Leu-OMe.[11] On the other hand, bovine prothrombin residues 1–39 do show an apparent tightening of coordination around Eu(III) in the presence of increasing concentrations of lanthanum(III) or gadolinium(III) ions.[11] The results reported here for the heptapeptide loop in the presence of lanthanum(III) ions suggest that slow metal ion exchange reflects to a major extent properties of the 17–23 region of the 1–39 structure.

Although a simple explanation of these exchange studies is not apparent from the available data, we believe that observed alterations in exchange rates are associated with the interaction of the metal ion binding sites present in this region of the polypeptide chain. An interesting manifestation of such interactions with consequences for the rate of prothrombinase-catalyzed activation of prothrombin to thrombin, has been reported by Prendergast and Mann.[7] In the presence of 1 m*M* Mg^{2+} ions, the Ca^{2+} ion concentration dependence of the prothrombin activation rate is shifted to lower calcium ion concentrations.[7] Explanations for such effects appear to require hypothese involving several classes of interacting sites. Rhee et al.[17] have presented high-resolution excitation scans of europium(III) bound to prothrombin, which suggest the existence of two classes of high-affinity europium ion binding sites on prothrombin with different environments. The shape of the europium ion saturation curves suggests that the sites fill cooperatively. Lanthanide ion binding to the heptapeptide loop may reasonably involve metal ion coordination-induced changes in at least one metal ion binding site's structure, thereby yielding a site at which the affinity for another metal ion is altered to a point at which exchange is slow relative to the excited-state lifetime of the species involved.

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(17) Rhee, M. J.; Horrocks, W. D., Jr.; Kosow, D. P. *Biochemistry* **1982**, *21*, 4524–4528.