A Bimetallic Helical Heptapeptide as a Transphosphorylation Catalyst in Water

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The realization of synthetic catalysts or molecular devices using properly assembled α-amino acid sequences to take advantage of the self-organization of polypeptides[1] in helices or *β*-sheet structures is quite appealing for the easy availability of the constituent building blocks.[2] Although considerable results have been recently reported using relatively long peptides,[3] shorter, and nevertheless conformationally stable sequences, would allow a more facile synthetic access to a wide variety of new peptide-based catalysts.

Recent studies by some of us[4] and other groups[5] have clearly shown that stable helical conformations can be elicited in very short sequences (ca. 7−8 amino acids, i.e., two helical turns) comprising an adequate number of C^{α}-tetrasubstituted α-amino acids. The typical conformation of these peptides is a 3_{10}-helix, slightly more stretched than an α-helix, and characterized by a pitch of 6.3 Å.[6] In this structure the side chains of two amino acids placed in $i/(i + 3)$ relative positions in the chain face each other on the same side of the helix. However, the onset of such a conformation has so far been confined to organic or mixed aqueous/organic solvents.[4,5,7]

We have recently reported[8] the synthesis of the azacrown-functionalized α-amino acid **1** which strongly coordinates transition-metal ions. We thought that incorporation of two copies of **1** in appropriate relative positions in a helix-forming peptide would allow one to take advantage of the cooperative effect between two metal centers in a catalytic process, such as that found in some metalloenzymes like, for instance, phosphatases and RNAses.[9] To this aim we have prepared heptapeptide **2** by solution-phase synthesis.[10] This peptide comprises five copies of the C^{α}-tetrasubstituted α-aminoisobutyric acid (Aib) with the assumption that it would be able to induce a stable helical conformation even to such a very short sequence.[4,5] Because of the presence of the two triazacyclononane rings in the side chains, peptide **2** is rather soluble in aqueous solution in the pH range 3−9 despite the lipophilicity of the five Aib residues. A circular dichroism (CD) study of **2** in pH 8 buffered aqueous solution (see Supporting Information) reveals the presence of a 3_{10}-helical structure, characterized by a negative Cotton effect at 206 nm accompanied by a pronounced shoulder centered near 222 nm.[11] *This is the first example of a short peptide assuming such a helical conformation in pure water*. Taking as the reference the published[11] CD spectrum of Ac[L-(α-Me)Val]$_8$-O*t*Bu [Ac, acetyl; (α-Me)Val, C^{α}-methyl valine; O*t*Bu, *tert*-butoxy] in 2,2,2-trifluoroethanol (TFE) solution, peptide **2** adopts the 3_{10}-helical conformation to a substantial extent under the above-mentioned experimental conditions. Addition of micellar cetyltrimethylammonium bromide (CTABr) or, even more, sodium dodecyl sulfate (SDS), causes an increase of the helical conformation. Interestingly, the peptide conformation is not affected either by added TFE or by coordination of transition-metal ions such as Cu(II). This latter evidence indicates that the two metal centers are not involved in helix stabilization in contrast to what reported for other peptides.[12] However, a decrease of the pH of the aqueous solution to 3.5 causes a significant decrease of the helicity likely because of the destabilizing repulsive interaction between the protonated nitrogens of the azamacrocycles.[13] A partial loss of helical conformation is also observed on heating (from 25 to 80 °C).

Peptide **2** binds up to two metal ions whose binding constants do not appear to be reciprocally influenced.[8] This also indicates that there is no formation of a sandwich-like complex with two macrocycles binding the same metal ion, in accord with the lack of any effect on the secondary structure of the peptide by metal ion coordination as mentioned above.

The potential utilization of the helical peptide scaffold for the exploitation of the synergic action of two metal centers was tested by using **2**·2Zn(II) in the catalysis of the intramolecular transesterification of the RNA model substrate 2-(hydroxypropyl)-*p*-nitrophenyl phosphate (HPNP). A comparison was made with the

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(13) The helicity increases upon increasing the pH from 3.5 to 6; thereafter it remains constant up to pH 9. This is in accord with the removal of one H^+ from each of the two diprotonated triazacyclononanes. The reported pK_a for this equilibrium for 1,4,7-triazacyclononane is 6.5, while the pK_a of the last proton is 10.4 (see: Smith, R. M.; Martell, A. E. *Critical Stability Constants*; Plenum: New York, 1989; Vol. 6.)

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Table 1. Kinetic Data[a] for the Complexes Studied at Different pHs and 40 °C

entry	catalyst	pH	$10^6\ k_\psi$, s^{-1}	entry	catalyst	pH	$10^6\ k_\psi$, s^{-1}
1	none	7.0[b]	0.39	5	**2**·2Zn(II)[c]	6.1[f]	12.0
2	**2**·2Zn(II)[c]	7.0[b]	21.0	6	**3**[c]	6.1[f]	3.5
3	**3**[d]	7.0[b]	7.0	7	**2**⊙2Zn(II)[c]	5.6[f]	5.6
4	**4**[e]	7.0[b]	2.5	8	**3**[d]	5.6[f]	1.2

[a] [HPNP] = 2×10^{-5} M; the cleavage process follows pseudo-first-order kinetics in all cases; the reported value for 0.5 mM Zn(II) is 4.7×10^{-6} s^{-1} (Breslow, R.; Berger, D.; Huang, D.-L. *J. Am. Chem. Soc.* **1990**, *112*, 3686). [b] 50 mM HEPES buffer. [c] 0.5 mM **2**, 1 mM Zn(II). [d] 1 mM. [e] 0.5 mM. [f] 50 mM MES buffer.

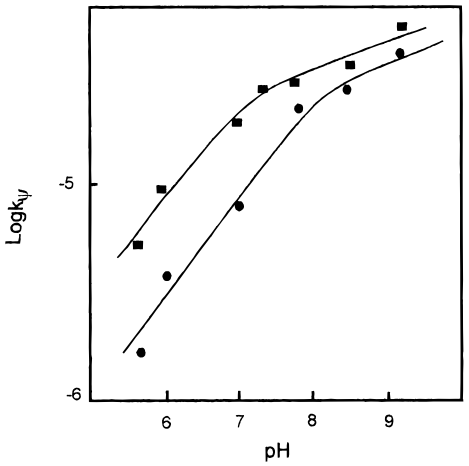

Figure 1. Log k_ψ vs pH profile for the cleavage of 2×10^{-5} M HPNP by 0.5 mM **2**·2Zn(II) (■) and 1 mM **3** (●) at 40 °C. Buffers used (50 mM) were: pH 5.6 and 6.1, MES; pH 7−8.1, HEPES; pH 8.5−9.2, CHES.

corresponding mononuclear Zn(II) complex of 1,4,7-triazacyclononane (**3**) and dinuclear Zn(II) complex of dipeptide $Ac\mathbf{1}_2$-OMe (OMe, methoxy) (**4**). This dipeptide lacks any specific conformation and indirectly provides evidence for the role of a defined secondary structure in affecting the mutual interaction between the two metal ions. Table 1 lists the results obtained with the different catalysts in 40 °C buffered aqueous solutions. The dinuclear complex of heptapeptide **2** shows clear evidence of cooperativity between the two metal ions being more active than mononuclear complex **3** (up to 5-fold rate acceleration at pH 5.6, compare entries 7 and 8). On the contrary, dinuclear complex **4** is *less* active than **3**[14](compare entries 3 and 4) so that the reaction in the presence of **2**·2Zn(II) is 1 order of magnitude faster than that with **4** as the catalyst. This extra activity, however, diminishes as the pH increases (compare entries 7 and 8, 5 and 6, 2 and 3) and eventually vanishes at pH >8 (see Figure 1). The dependence of the observed rate constants from the pH for reference complex **3** reported in Figure 1 indicates that the pK_a of the nucleophilic species (likely Zn(II)-bound H_2O) is ca. 7.8.

A reasonable mechanism accounting for the cooperativity[15,16] between the two Zn(II) ions in **2**·2Zn(II) is shown in Figure 2. It

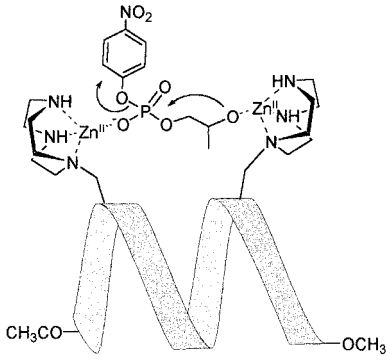

Figure 2. Proposed mechanism for the cleavage of HPNP by **2**·2Zn(II).

requires the binding of the phosphate to one metal ion while the second Zn(II) ion activates the hydroxyl group for intramolecular nucleophilic attack.[17] The involvement of this hydroxyl in the process is strongly supported by the failure of **2**·2Zn(II) to accelerate the cleavage of a phosphodiesters devoid of such a group like bis-*p*-nitrophenylposphate. Molecular models show that the distance between the P−O$^-$ and the hydroxyl of HPNP is about 5 Å so that the molecule fits nicely within the two Zn(II) ions which are placed at a distance of ca. 6.3 Å, the pitch of the helix, if one allows for the coordination to the metal centers. The flexibility of the lateral arms bearing the azamacrocycles allows the occurrence of the intramolecular attack at the phosphorus without complete loss of coordination of the hydroxyl.

The coordination of the substrate to Zn(II) is likely pH-dependent since it needs the displacement of the fourth ligand (water) from the metal: this is easier with H_2O than with OH^-.[16] Accordingly, at pH > 8 when the water molecule bound to Zn-(II) is fully deprotonated, the binding is inhibited and the reaction follows a mechanism similar to that operative with mononuclear complex **3**. Reactions carried out in the presence of different **2**·2Zn/HPNP ratios at pH 7 and 40 °C show clear tendency toward saturation and allow the determination of $K_b = 250 \pm 20$ M^{-1} for the 1:1 complex of the substrate with the Zn(II) heptapeptide and a k_{lim} for the fully bound substrate[18,19] of 7×10^{-5} s^{-1}.

In conclusion, heptapeptide **2** provides the first example of a very short peptide highly organized in a 3_{10}-helical conformation in neutral aqueous solution. Its dinuclear complex with Zn(II) is a catalyst of the intramolecular transesterification of HPNP, a RNA model substrate, with a clear indication of cooperativity between the two Zn(II) ions in the catalytic process. Hence, we succeeded in demonstrating that very short, easily accessible, economically synthesized, and rationally designed peptide sequences may provide the appropriate scaffold for the realization of effective catalysts by controlling their secondary structure.

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Supporting Information Available: Experimental details for the synthesis and characterization of peptide **2** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Very likely this is related to the partial formation of an inactive, sandwich-like complex. This conclusion is reasonable in view of the lack of any conformational constraint for this dipeptide in contrast to what is observed for the heptapeptide.

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(19) However, the acceleration due to the cooperation between the two Zn(II) ions is not fully exploited because the binding constants of HPNP to **2**·2Zn(II) and **3** are quite similar, and hence the substrate can also interact with the metal from the side where it cannot take advantage of the interaction with the second Zn(II). A more sophisticated catalyst would require a specific recognition site for the substrate in order to direct it in the correct position for the interaction with the two metal ions.[16]