Lanthanide Complexes of Asp-peptides. Models for Calcium Binding Sites*

HAMID ZINEDDINE, MARCEL ASSO† and DAVID BENLIAN

Université de Provence, Laboratoire de Chimie de Coordination, St-Jerôme, Ave H. Poincare, 13397 Marseille Cédex 13, France

Experimental

Two peptides have been synthesized by a solid-state method with similar reactants and procedure, as already described in a previous report [1]. The sequences were chosen so that the role of the side chains could be investigated: YVDA and DGYVDA. Moreover, these simple peptide sequences may represent a part of the calcium binding site of troponin C [2] or thymopoietin [3].

Purification was carried out on a Sephadex G15 column in 0.1 M ammonium acetate. Analyses of the samples have given the following values:

	Tyr	Val	Asp	Ala	Gly
YVDA	0.89	1.00	0.98	1.02	
DGYVDA	0.90	1.00	1.99	1.03	0.87

Stock solutions of YVDA or DGYVDA were prepared by adding solid peptide to H_2O containing KCl (0.5 M) for pH titration and D_2O KCl (0.5 M) for NMR measurements. Solutions were bubbled with argon before titration and bathed with argon during titration to minimize absorption of CO_2. pH measurements were made at 25 °C with a Metrohm E603 pH-meter equipped with a Metrohm EA 120 microcombination electrode. ^1H NMR spectra were obtained at 200 MHz and 25 °C with a Bruker WH-200 spectrometer operating in the pulse/Fourier transformer mode.

The concentration of peptides (C_L) was maintained constant (10^{-2} M) and to each sample was added an aliquot of Ln^{3+} (Ln = lanthanide) cation solution to achieve a final concentration of C_M. A series of spectra with $0 < C_M/C_L < 5$ constitutes the experimental data for the β value calculation and for the conformation fitting.

*Paper presented at the Second International Conference on the Basic and Applied Chemistry of f-Transition (Lanthanide and Actinide) and Related Elements (2nd ICLA), Lisbon, Portugal, April 6–10, 1987.

†Author to whom correspondence should be addressed.

Results and Discussion

The computation is based on the values of the bound shifts [4] for the protons in the peptide molecule (Figs. 1 and 2). The details of the fitting procedure have been described for the case of DVDA and of aspartame [5]. Protons of the ligand molecule have been located in space around the paramagnetic cation by a simulation procedure which was fitted until convergence was obtained with the minimum value for D and V convergence tests. D is the difference $|R_{est} - R_{cal}|$ with R_{est} being the distance from the lanthanide atom and C atom of the carboxylic group for an Ala or an Asp residue estimated 2.5 Å $< R_{est} <$ 3.5 Å. V is the agreement factor.

The starting conformations were obtained by combining the 5^4 possible forms (Φ and ϕ standard data) with those given by the rotamer distribution.

Convergence was observed only in a few cases for the following rigid-lock situations:

pD = 2.5

YVDA–M (M = Pr^{3+}, Dy^{3+})

$\alpha_R\alpha'_R\alpha_L\beta''$: bond length M–$C^0$(Ala) = 3.0 ± 0.1 Å.

DGYVDA–M

$\alpha_R\alpha_R\alpha_R\alpha'_R\alpha_L\beta''$: bond angle M–$C^0$–$C_\alpha$(Ala) = 140 ± 10°.

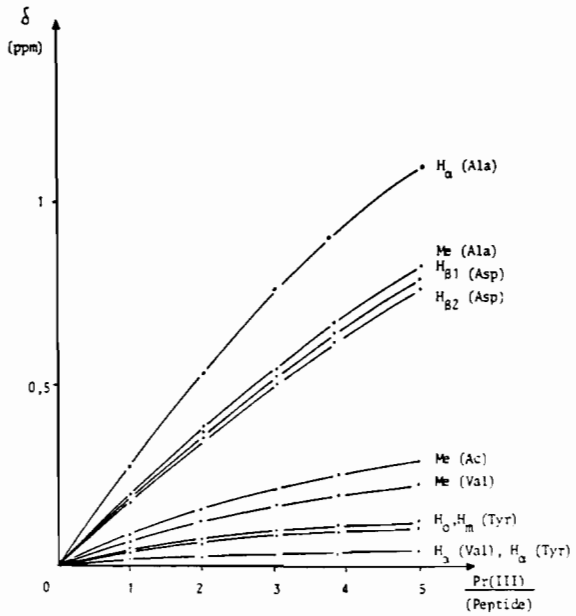

Fig. 1. Variation of chemical shifts of ^1H nuclei of YVDA as a function of added $PrCl_3$ (pD = 2.5).

0020-1693/87/$3.50

TABLE I: Stability Constants β_{pqr} for different Asp-peptide Complexes Established by Potentiometric Measurements

	Ca	Mn	Cu	Zn	Dy	Pr
Ac–YVDA						
log β_{121}					0.70*	0.75*
log β_{111}	2.44	2.01	2.68	2.58	2.48	2.50
log β_{101}	3.04	3.10	5.54	4.66	3.11	3.15
Ac–DGYVDA						
log β_{131}					0.75*	0.78*
log β_{121}	1.93	2.04	2.30	1.01	2.42	2.47
log β_{111}	3.07	3.08	4.24	2.89	3.09	3.13

*Found by ^{1}H NMR shifts.

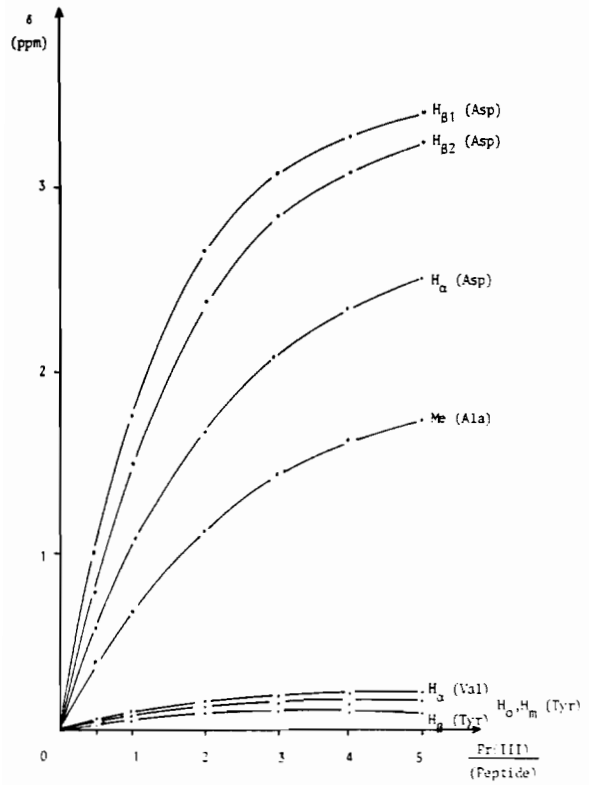

Fig. 2. Variation of chemical shifts of ^{1}H nuclei of YVDA as a function of added $PrCl_3$ (pD = 5.5).

pD = 5.5

YVDA–M

$\alpha_L\alpha_R\alpha_L\beta$: bond length M–$C_\gamma$(Asp) = 2.90 ± 0.1 Å.

$\beta''\beta''\alpha_R\beta$: bond angle M–$C_\gamma$–$C_\beta$(Asp) = 135 ± 10°.

At low pH, the side chains are protonated and the Ala carboxylate is the unique complexing site. In this model Pr(III) should interact with the carboxylate group of the alanine C terminal.

When the pH is raised, a noticeable shift is observed in the Asp side-chain resonances, which denote its folding towards the Ln cation.

Argument for a preferential Ln–Asp interaction is consistent with the value of the stability constant β_{101}, which is higher than in a monodentate but lower than in the bidentate chelate in which two carboxylic residues are folded in the most efficient geometry [1] (Table I). As both g_I and g_{II} rotamers are present in equal populations, no unique conformation can be proposed for the Asp side chain.

This effect on the actual protein binding site could be due to a cooperative action of the whole polymer. In the simple models presented here, it appears that Tyr side chains lay extended away from the cation site. This conformation does not seem to result from specific interactions of the numerous vicinal residues in a larger peptide or in the protein. Even in a very simplified structure, the position adopted by Tyr is favorable to the hydrophobic action proposed by Lee and Sykes [6], giving a more stable complex with Ca(II).

References

1 M. Asso, C. Granier, J. Van Rietschoten and D. Benlian, *Int. J. Pep. Res., 26*, 10 (1985).
2 R. A. Weeks and S. V. Perry, *Biochem. J., 173*, 449 (1978).
3 J. B. Vaughn, R. L. Stephens, R. E. Renkinski, N. R. Krishima, G. A. Heavner and G. Goldstein, *Biochim. Biophys. Acta, 671*, 50 (1981).
4 J. Mossoyan, M. Asso and D. Benlian, *J. Magn. Reson., 55*, 188 (1983).
5 M. Asso, H. Zineddine and D. Benlian, *Int. J. Pep. Res., 28*, 437 (1986).
6 L. Lee and B. D. Sykes, *Biochemistry, 22*, 4366 (1983).