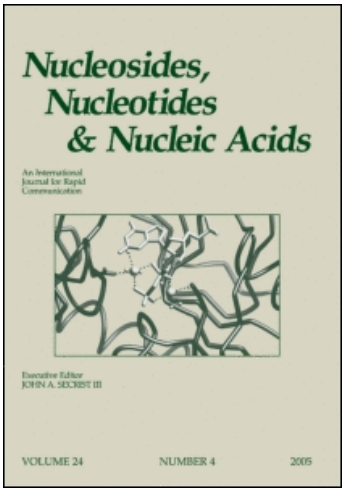

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Calorimetric Evidences for Copper(II)-Regulated Chiral Recognition Between Decanucleotide 5'd(CTGGATCCAG)$_2$ and ALA-TRP Dipeptides

Vincenzo Cucinotta[a]; Domenico Grasso[b]; Giulia Grasso[c]; Danilo Milardi[b]
[a] Dipartimento di Scienze degli Alimenti, Università Federico 11, via Università, Napoli, (Italy) [b] Dipartimento di Scienze Chimiche, Università degli studi di Catania, Viale Andrea Dona, (Italy) [c] Istituto per lo Studio delle Sostanze Naturali di Interesse Alimentare e Chimico Farmaceutico del C.N.R., Viale Andrea Dona, Catania, (Italy)

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CALORIMETRIC EVIDENCES FOR COPPER(II)-REGULATED CHIRAL RECOGNITION BETWEEN DECANUCLEOTIDE 5'd(CTGGATCCAG)$_2$ AND ALA-TRP DIPEPTIDES.

Vincenzo Cucinotta[a], Domenico Grasso[b], Giulia Grasso[c] and Danilo Milardi*[b].

[a] Dipartimento di Scienze degli Alimenti, Università Federico II, via Università, 100 - 80055 Portici, Napoli (Italy).

[b] Dipartimento di Scienze Chimiche, Università degli studi di Catania, Viale Andrea Doria, 6 - 95125 (Italy).

[c] Istituto per lo Studio delle Sostanze Naturali di Interesse Alimentare e Chimico Farmaceutico del C.N.R., Viale Andrea Doria, 6 - 95125 Catania (Italy).

ABSTRACT

DSC measurements of binary, decanucleotide/L,L or L,D Ala-Trp, and ternary decanucleotide/L,L or L,D Ala-Trp/Copper(II), systems have been carried out. The results obtained show the different behaviour of the two diastereoisomers both in the binary and in the ternary systems, ascribable to the different disposition of the side-chains in the dipeptides.

INTRODUCTION

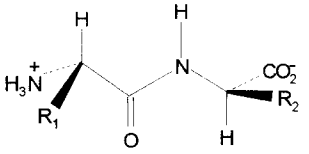
Almost every aspect of complex biological systems is controlled by processes that, from a biochemical point of view, rely on molecular recognition phenomena. Ability of individual molecules to bind selectively to structurally and functionally related target systems is a key principle for understanding biochemical reactivity. Numerous biophysical chemistry research projects have been undertaken with the aim of determining molecular recognition phenomena in thermodynamic terms, especially in the case of phenomena involving the interaction of small molecules with highly complex polymers[1].

One of the most intriguing problems concerns the mechanisms controlling protein-DNA recognition[2,3]. Mechanisms that regulate the chiral recognition of biological molecules have been the focal point of intense research over the last few decades[4]. In addition, the influence of metal ions (copper (II) in particular), which are believed to play an essential role in the correct functioning of these complexes, is still not completely understood[5]. In order to circumvent the difficulties inherent in these systems, model compounds (nucleotides - peptides) have been extensively investigated using both theoretical and experimental approaches[6-11].

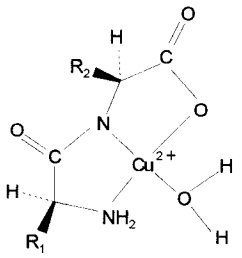
Differential Scanning Calorimetry (DSC) is an effective tool in the evaluation of the "energetics" that control molecular interactions. In the last few years DSC measurements have provided a wealth of thermodynamic information about DNA and self-complementary DNA oligomers[12-15]. However, very little thermodynamic data are available in literature about peptide -oligonucleotide interactions.

To clarify the role played by copper(II) in chiral recognition between dipeptides and oligonucleotides, DSC experiments were carried out on two-component (peptide-oligonucleotide) and three-component (peptide-copper(II)-oligonucleotide) complexes.

The oligonucleotide studied here is a self-complementary DNA decamer 5'd(CTGGATCCAG)$_2$ (ODN), comprising the specific target site for the restriction endonuclease *Bam*H1 [16]. In previous research where the complexing features of diastereoisomeric pairs of dipeptides have been studied by using a thermodynamic and spectroscopic approach[24], it has been shown that the disposition of the dipeptide side-chains may change according to the diastereoisomer in question. In uncomplexed dipeptides, the side-chains are found to be on the same side in the L,D-diastereoisomer. In the amide-deprotonated copper(II) complex, the dipeptide behaves as a tridentate ligand and is forced to rotate (see the schemes reported below). As a consequence, its side-chains are found on the same side in the L,L-diastereoisomer. By comparing this difference it is possible to underline the influence that side-chain disposition exerts on the interaction with the oligonucleotide. In this paper, the dipeptide pair chosen was Ala-Trp, in order to obtain information concerning how the indole moiety influences ODN.



L-Ala-D-Trp



L-Ala L-Trp / Copper (II) complex

EXPERIMENTAL

Synthesis and purification of 5'd(CTGGATCCAG)$_2$

The decanucleotide was synthesized by Cyclone Biosearch (1 μm column) on a solid support of controlled-pore glass using the β-cyanoethyl phosphoramidite method. Upon completion of chain elongation, the oligomer was removed from the solid support and the protecting groups were removed by treatment with concentrated ammonia. The oligonucleotide was purified by HPLC chromatography with an anion-exchange column (Partisil-10-SAX, 10 μ m, 250X9 4 mm, Whatman) eluting at 4 ml min^{-1} with a phosphate buffer, pH 6.5, in 5% EtOH (linear gradient from 0.001 to 0.4 M in 40 min.) at room temperature. The pure product was desalted by flash chromatography with a reverse phase column (LiChroprep RP-18 25-40 m, Merck, ϕ = 2 cm, h = 10 cm) using first water and then H_2O/ CH_3CN- 95:5 as an eluent. Purity was checked by HPLC with an Ultrasphere ODS column (5 μm, 150x4.6 mm; Beckman), using a linear gradient going from 100% ATEA (0.1M in water solution) to 70% ATEA (0.1M in water solution)-30% CH_3CN in 40 min. at 1 ml/min. Decanucleotide concentration was determined by UV absorbance measurements (260 nm) using a molar extinction coefficient of 172000 $M^{-1}cm^{-1}$ (double strand) [18].

Chemicals

L-Ala-L-Trp was purchased from BACHEM L-Ala-D-Trp were synthesized as described[15] elsewhere. Reagents for decanucleotide synthesis were purchased from Milligen/Biosearch.

Thermodynamic measurements

Differential Scanning Calorimetry

DSC scans were carried out with a SETARAM (Lyon, France) micro differential scanning calorimeter (microDSC) with stainless steel 1 ml sample cells, interfaced with a BULL 200 Micral computer. The sampling rate was 1

point/second in all measuring ranges. The decamer (5 mg/ml) was dissolved in 5 mM phosphate buffer at pH= 6.7. Ionic strength was adjusted at 0.1 M by Sodium Chloride. The same phosphate buffer was used in the reference cell. In each experiment, the oligonucleotide concentration was kept constant at a 1:20 (base pairs) ODN/dipeptide and 1:20:20 (base pairs) ODN/ dipeptide/copper(II) ratio. In every experiment the sample volume of 0.2 ml was used. Both sample and reference were scanned from 20 to 100°C with a precision of ±0.08 °C, at the scanning rate of 0.5 °C/min. The calorimetric scans were carried out under an extra nitrogen pressure of 1.1 bar.

For the Cp curves, buffer-buffer base lines were obtained at the same scanning rate and then subtracted from sample curves[19,21]. The excess specific heat capacity (Cp_{exc}) is the amount by which the apparent specific heat curve exceeds the baseline during a DSC transition involving the solute. All of the Cp_{exc} curves were obtained using a fourth-order polynomial fit as baseline[21]. Average noise level was about ±0.4 μW and reproducibility at refilling was about 0.1 mJ/K/ml.

Calibration in energy was obtained by providing a definite power supply, electrically generated by an EJ2 SETARAM Joule calibrator within the sample cell.

RESULTS AND DISCUSSION

In FIGURE 1 the DSC transition of ODN, in the 25 - 90 °C range is reported. The overall profile of the thermogram is analogous to those previously reported for oligonucleotides of different sequences[13-16]. The dotted curve represents a baseline obtained as reported in the experimental section. All calorimetric scans were reversible, i.e. a second run of a previously scanned sample shows a signal very similar to the first one. In FIGURE 2 we report the Cp_{exc} curves (in kJ per mole of base pairs) for the following systems: the ODN (curve a), ODN/ L-Ala-L-Trp (curve b), ODN / L-Ala-D-Trp (curve c). As can be seen in the curves shown in FIGURE 2 and the corresponding data in TABLE 1, when L-Ala-L-Trp is added to the decamer (see curve b), melting temperature, T_m, (defined as the temperature corresponding to the maximum value of Cp_{exc}[13]) and enthalpy both change alongside the conformational transition in the decanucleotide (ΔH=622 KJ/mbp). An endothermic effect shown as a shoulder on the left side of the Thermogram is also located at about 48 °C.

When L-Ala-D-Trp is added to the solution containing the ODN, the effect on the calorimetric curve is more evident compared to that referring to the ODN alone (curve c). In fact, the denaturational enthalpy of 274 kJ/mbp is obtained, and the melting temperature shifts at 59.2 °C. An endothermic effect located at about 42 °C is also evident, and its relative intensity compared to the major transition is more noticeable than in the case of curve b. To get a clearer picture of the endothermal effects involved in the DSC transitions (see curve b and c of FIGURE 2) it was decided to fit these experimental curves with two gaussians applying the SIMPLEX minimization algorithm[22]. When using the best fit procedure for the deconvolution of calorimetric data, one should bear in mind that the closeness of the experimental heat capacity curve to the calculated one improves with an increase in the number of states (or gaussians) considered. However, this improvement slows significantly beyond a definite number of states[23]. In this study, we chose the number of states so that the experimental curve coincided with the calculated curve within the accuracy limits of the experiment. We found that two gaussians satisfied this condition in all cases considered. The parameters of the deconvoluted gaussians are reported in TABLE 1.

As already indicated in the introduction, some metal ions are assumed to play a key role in peptide - nucleotide interactions. In the light of this consideration, we investigated the effect of copper(II) on the thermal transition of ODN/L-Ala-L-Trp or L-Ala-D-Trp complexes.

When added to the decamer copper(II) has no influence on the calorimetric curve, which is practically identical to the DSC curve for the transition of the pure decamer. However the effect of copper is evident when added to a complex

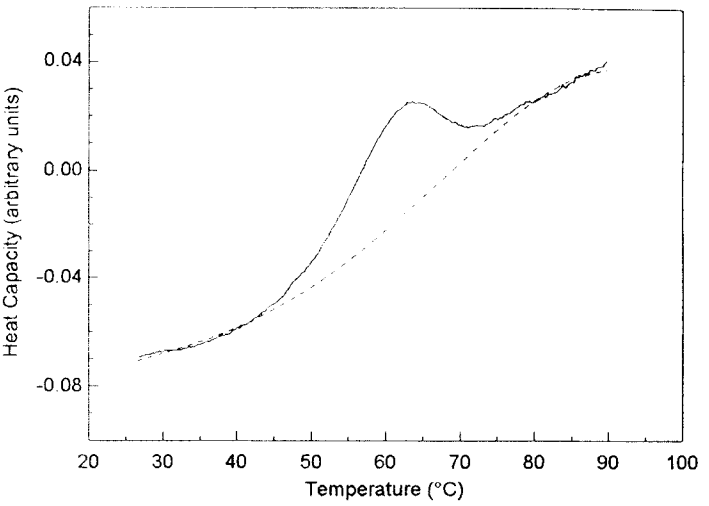


Figure 1. DSC thermogram of ODN after subtraction of the buffer-buffer base line. DNA decamer concentration was about 5 mg/ml, pH=6.7, ionic strength 0.1 M in NaCl, scan rate=0.5 °C/min. The base line (dashed curve) was obtained as described in the text.

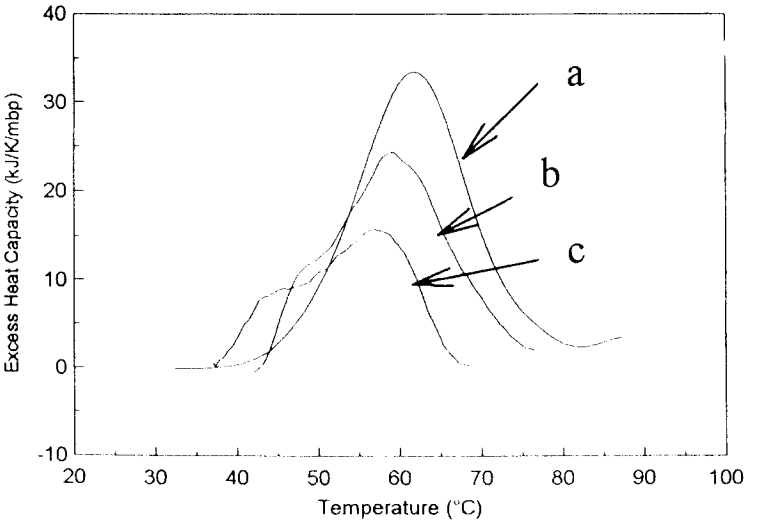


Figure2 . Effect of the interactions between ODN and dipeptide L,Ala-L,Trp (curve b) and L,Ala-D,Trp (curve c) on the excess heat capacity function of ODN. Curve a represents the excess heat capacity function of ODN without any dipeptide. Scan rate was 0.5 °C/min in all experiments.

Table 1. Calorimetric data concerning the thermal transitions of ODN, ODN/dipeptide and ODN/dipeptide/copper (II).

[a]Transition enthalpies (ΔH), calculated by integration of excess heat capacity functions, are expressed as mean ± standard deviation. [b] The estimated inaccuracy of temperatures is ± 0.1 °C. Enthalpy changes were calculated by integration of the Cp_{exc} curves. Complex transitions were fitted by two Gaussians (see text for details).

	ΔH^a (kJ/mbp)		$T_m{}^b$ (°C)	
ODN	622 ± 34		61.8	
ODN/L,L AlaTrp	451 ± 25		59.0	
(the two separate components)	24 ± 12	415 ± 18	47.7	58.5
ODN/L,D AlaTrp	274 ± 28		59.2	
(the two separate componets)	96 ± 7	181 ± 28	46.1	57.3
ODN/L,L AlaTrp/Cu(II)	434 ± 32		59.3	
ODN/L,D AlaTrp/Cu(II)	180 ± 26		58.4	

decamer-dipeptide. FIGURE 3 shows the Cp_{exc} curves for the thermal transition of the binary system ODN/L-Ala-L-Trp (curve a) and the ternary system ODN/L-Ala-L-Trp/copper(II) (curve b). When copper is present the shoulder on the left side of the thermogram disappears. This shows that copper(II) allows the ODN to partially recover its initial thermal behaviour (in terms of the number of gaussians fitting the curve). The other calorimetric parameters, T_m and ΔH, do not change appreciably.

Similar behaviour was shown for ODN/L-Ala-D-Trp (FIGURE 4 curve a) and ODN/L-Ala-D-Trp/copper(II) (FIGURE 4 curve b) respectively. In fact, the shoulder of curve a disappears when copper(II) is added to the system. By comparing FIGURE 3 with FIGURE 4, it becomes clear that the enthalpic changes in the two cases are quite different. For the binary complex ΔH is 274 kJ/mbp, while for the system where copper is added to the decanucleotide-dipeptide solution ΔH is only 180 kJ/mbp. On the other hand, melting temperatures in the two cases are not appreciably different. When compared to the decanucleotide only, the enthalpy change associated with decanucleotide melting in ternary systems is seen to be less negative. This corresponds to an analogous decrease in melting temperature, thus showing that enthalpy is, in fact, the driving force in such processes.

Our results seem to suggest that the formation of these complexes destabilizes the oligonucleotide in its double strand form. It is still not clear, however, why a complex should form if the stability of the system decreases.

Nevertheless, we must remember that enthalpy change is the difference between a final and an initial state.This can be summarised in a formula as follows:

$\Delta H_t = H_{f,t} - H_{i,t}$

$\Delta H_d = H_{f,d} - H_{i,d}$

where ΔH_t is the enthalpy change in the ternary system; ΔH_d is the enthalpy change in the decanucleotide; $H_{f,t}$ is the enthalpy of the ternary system in the final state; $H_{i,t}$ is the enthalpy of the ternary system in the initial state; $H_{f,d}$ is the enthalpy of the decanucleotide in the final state; and $H_{i,d}$ is the enthalpy of the decanucleotide in the initial state.

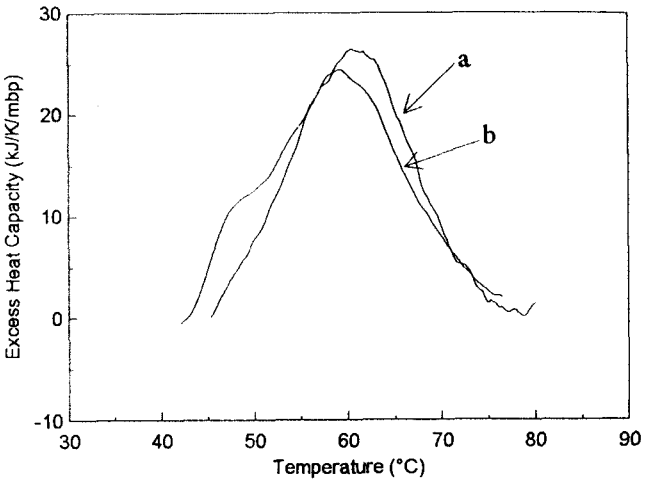


Figure 3. Effect of Copper (II) on the excess heat capacity function of ODN/L,L AlaTrp dipeptide binary systems. Curve a reports the excess heat capacity function of the system ODN/L,Ala-L,Trp/copper. Curve b represents the excess heat capacity function of the system ODN/L,Ala-L,Trp.

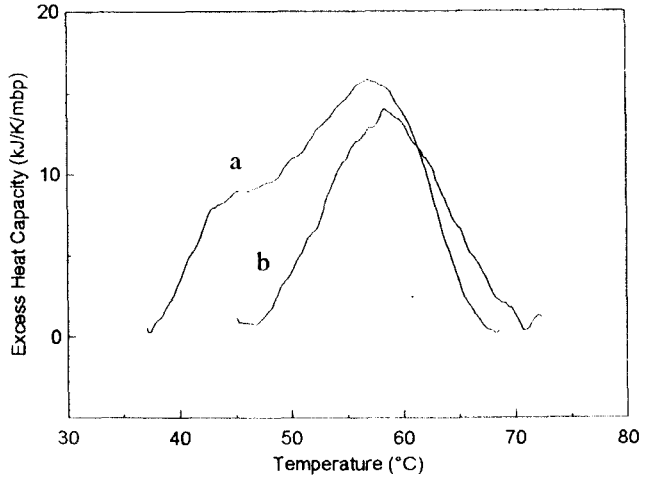


Figure 4. Effect of Copper (II) on the excess heat capacity function of ODN/L,D AlaTrp dipeptide binary systems. Curve a reports the excess heat capacity function of the system ODN/L,Ala-D,Trp/copper. Curve b represents the excess heat capacity function of the system ODN/L,Ala-D,Trp.

Thus, if, $H_{i,t}$ cannot be higher than $H_{i,d}$, then in order to have :

$\Delta H_t > \Delta H_d$

it is necessary that:

$H_{f,t} - H_{f,d} < H_{i,t} - H_{i,d}$

This means that the final state of the ternary system, with the presumable formation of a ternary complex involving the oligonucleotide single strand, the dipeptide and the copper (II) ion, is more stable in comparison with the analogous double strand ternary system which corresponds to the initial state.

The calorimetric analysis reported shows the role played by stereochemistry and/or copper(II) in the "energetics" of peptides-nucleotide interactions.

Points of interest may be summarized as follow: a) The DSC transition relative to ODN is ascribable to a single reversible cooperative process. The cooperative unit of this transition, calculated as previously suggested[14,15], is about 0.5 in accordance with the results obtained for similar systems[13]. b) Binary systems ODN/L-Ala-L-Trp and ODN/L-Ala-D-Trp show different thermal behaviour. The difference between the two systems, shown for the first time by means of DSC techniques, is dependent on the stereochemistry of the dipeptide. c) Copper(II) influences the overall thermal stability of binary systems in a different way.

The calorimetric curves reported in the present paper appear to confirm that the effect of copper(II) is also controlled by the configuration of the dipeptide. In fact, in the case of the ternary system with the L,L diastereoisomer the presence of copper(II) does not influence ΔH and T_m significantly. In contrast, for the ternary system with the L,D diastereoisomer the disapperarance of the minor shoulder of the peak is accompanied by a decrease in ΔH.

The data reported in TABLE 1 appear to show a decrease in ODN double strand stability for each system, as shown by the decrease of both T_m and ΔH. However, it cannot be excluded that this decrease is due to the increase in single strand stability which may result from the formation of binary and ternary complexes with dipeptides and/or copper (II) at this stage.

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REFERENCES

1) W. Saenger, *Principles of Nucleic Acid Structure*, 1984, Springer-Verlag, New York.

2) P. H. Von Hippel and J.D.McGher, *Annu. Rev. Biochem.*, 1972, **41**,231.

3) C. Helene and J. C. Maurizot, *CRC Crit. Rev. Biochem.*, 1981, **10**, 213.

4) R.R. Sinden, *DNA Structure and Function*,1994, Academic Press, San Diego.

5) I. Samasundaram, M.K. Kommiya and M. PalaniandaVAR, *J.Chem.Soc.Dalton Trans.*, 1991, 2083.

6) E.J.Gabbay, P.D. Adawadkar, L.Kapicak, S. Pearce and W.D. Wilson, *Biochemistry*, 1976, **15,** 152.

7) R.D. Sheardy and E.J. Gabbay, *Biochemistry*, 1983, **22,** 2061.

8) E.J.Gabbay, P.D. Adawadkar and W.D.Wilson, *Biochemistry*, 1976, **15,** 146.

9) P.D.Adawadkar, W.D.Wilson, W. Brey and E. J. Gabbay, *J. Am. Chem. Soc.*, 1975, **97,** 1959.

10) E.J.Gabbay, K. Sanford, C.S. Baxter and L. Kapicak, *Biochemistry*, 1973, **12**, 4021

11) E.J.Gabbay, K. Sanford and C.S. Baxter, *Biochemistry*, 1972, **11**, 3429.

12) L.A. Marky and K.J. Breslauer, *Biopolymers*, 1982, **21,** 2185.

13) D.J. Patel, S.A. Kozlowski, L.A. Marky, J.A Rice, C. Broka, K. Itakura and K.J. Breslauer, *Biochemistry*, 1982, **21,** 451.

14) L.A. Marky, L. Canuel, R.A. Jones and K.J. Breslauer, *Biophysical Chemistry*, 1981, **13,** 141.

15) K.J. Breslauer, in Hans-Jurgen Hinz (Ed.), *Thermodynamic data for Biochemistry and Biotechnology*, 1986, Springer-Verlag Berlin, New-York.

16) M. Nilges, G.M. Clore, A.M. Groneborn, N. Piel and L.W. McLaughlin, *Biochemistry*, 1987, **26,** 3734.

17) V. Cucinotta, G. Grasso, G. Maccarrone, L. Mastruzzo and G. Pappalardo, *Inorg. Chim. Acta*, 1995, **228,** 119.

18) R. P. Bonomo, V. Cucinotta, G. Grasso, G. Maccarrone and L. Mastruzzo, 1996, *Inorg. Biochem.*, submitted.

19) J.M. Sturtevant, *Annu. Rev. Phys. Chem.*, 1987, **38**, 463.

20) P. Connelly, L. Ghosaini, C.Q. Hu, S. Kitamura, A. Tanaka and J.M. Sturtevant, *Biochemistry*, 1991, **30,** 1887.

21) C. La Rosa, D. Milardi and D. Grasso, *J. Phys. Chem.*, 1995, **99,** 14864.

22) J.A. Nelder and R. Mead, Comput. J., 1965, **7,** 398.

23) D. Milardi, C. La Rosa and D. Grasso, *Thermochim. Acta*, 1994, **246,** 183.

24) V. Cucinotta, R. Purrello and E. Rizzarelli, *Comments Inorg. Chem.*, 1990, **11,** 85.

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