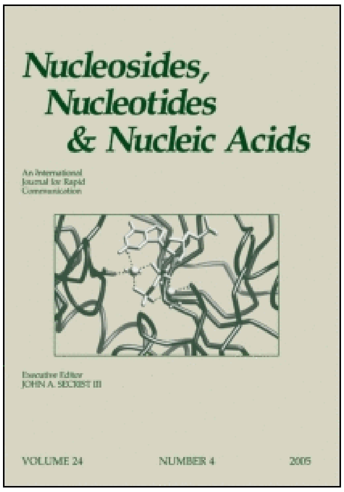

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Thermodynamics of Interaction of Phthalocyanine-Oligonucleotide Conjugates with Single- and Double-Stranded DNA

A. A. Chernonosov[a]; N. A. Kuznetsov[a]; V. V. Koval[a]; D. V. Pyshnyi[a]; N. M. Derkacheva[b]; E. A. Lukyanets[b]; O. S. Fedorova[a]
[a] Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia [b] Organic Intermediates and Dyes Institute, Moscow, Russia

To cite this Article Chernonosov, A. A. , Kuznetsov, N. A. , Koval, V. V. , Pyshnyi, D. V. , Derkacheva, N. M. , Lukyanets, E. A. and Fedorova, O. S.(2004) 'Thermodynamics of Interaction of Phthalocyanine-Oligonucleotide Conjugates with Single- and Double-Stranded DNA', Nucleosides, Nucleotides and Nucleic Acids, 23: 6, 983 — 987

To link to this Article: DOI: 10.1081/NCN-200026051

URL: http://dx.doi.org/10.1081/NCN-200026051

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 6 & 7, pp. 983–987, 2004

Thermodynamics of Interaction of Phthalocyanine-Oligonucleotide Conjugates with Single- and Double-Stranded DNA

**A. A. Chernonosov,[1] N. A. Kuznetsov,[1] V. V. Koval,[1] D. V. Pyshnyi,[1]
N. M. Derkacheva,[2] E. A. Lukyanets,[2] and O. S. Fedorova[1,*]**

[1]Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia
[2]Organic Intermediates and Dyes Institute, Moscow, Russia

ABSTRACT

Thermodynamics of interaction of phthalocyanine-oligonucleotide conjugates with single- and double-stranded DNA resulting in formation of duplexes and triplexes was measured by UV melting method. It was shown that a phthalocyanine moiety of conjugates stabilized the formation of duplexes and triplexes.

Key Words: Phthalocyanines; PDT; Oligonucleotide conjugates; DNA duplexes and Triplexes; Thermodynamics.

INTRODUCTION

Phthalocyanines (Ptc) represent an interesting family of compounds used now for the photodynamic therapy (PDT) of malignant tumors.[1] They can initiate DNA oxidation acting as photosensitizers for the generation of singlet molecular oxygen. In addition, complexes of phthalocyanines with ions of transition metals (Fe, Co, etc.) can

---

[*] Correspondence: O. S. Fedorova, Institute of Chemical Biology and Fundamental Medicine, Lavrentyev Ave. 8, Novosibirsk, 630090, Russia; E-mail: fedorova@niboch.nsc.ru.

DOI: 10.1081/NCN-200026051 1525-7770 (Print); 1532-2335 (Online)

www.dekker.com

catalyze formation of reactive oxygen species (ROS): O_2^-, H_2O_2, and OH.[2,3] These species are capable of damaging various cell constituents including DNA. Thus, these properties of metallophthalocyanines make them very attractive as reactive groups to be linked to antisense oligonucleotides for specific DNA modification.

RESULTS AND DISCUSSION

Phthalocyanines are large, planar, and hydrophobic molecules (Fig. 1). They may display an increased affinity to nucleic acid bases. The presence of phthalocyanine moieties in oligonucleotide conjugates can stabilize double- and triple-stranded DNA complexes.

In this paper we describe the thermodynamic study of interactions of phthalocyanine conjugates with DNA chains resulting in formation of duplexes and triplexes. The conjugate of Co(II)tetracarboxyphthalocyanine (Co(II)-Ptc) (Fig. 1) with deoxyribonucleotide pd(TCTTCCCA) **1** was synthesized according to procedure described in our previous works.[4,5] Oligonucleotide **1** and conjugate **2** can form with oligonucleotide target **3** the duplexes **4** and **5**, with target **6** triplexes **7** and **8**, and with target **9** triplexes **10** and **11**, respectively. Targets **3**, **6** and **9** contain the specific binding site *T*GGGAAGA. Complexes **4**, **7** and **10** do not contain a phthalocyanine moiety, while complexes **5**, **8** and **11** have one. The triplex-forming oligonucleotides **6** and **9** adopt the hairpin structures.

The thermodynamic characteristics of DNA complexes with or without phthalocyanine moieties were calculated using UV melting curve data.

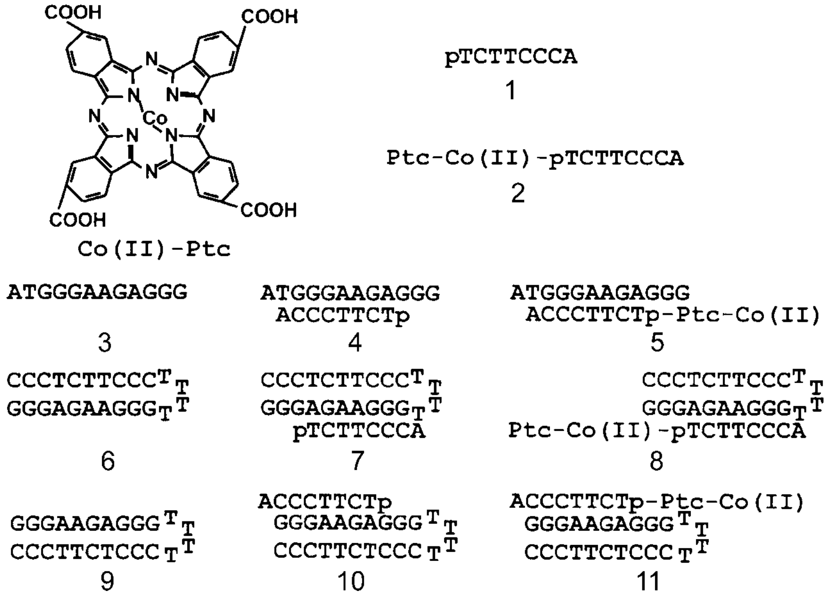

Figure 1. Structures of Co(II)-phthalocyanine, oligonucleotides and their complexes.

Experiments with structures **2–5** were conducted in a buffer containing 0.16 M NaCl, 0.02 M NaH_2PO_4 (pH = 7.5), 1 mM Na_2EDTA adjusted to pH 7.5, and with structures **6–11** in 0.1 M NaCl, 0.005 M $MgCl_2$, 0.01 M cacodylate adjusted to pH 5.33.

UV MELTING CURVES

Absorbance versus temperature profiles were recorded at 260, 270, 280, 300 and 360 nm by using the optical detector of a chromatograph ''Milikhrom'' (Nauchpribor, Orel, Russia) coupled with a PC. Melting profiles were obtained by increasing the temperature of 0.5–0.9 °C per minute. Each strand concentration was 5×10^{-6} M and the cell volume was 2 μl. The data were analyzed taking into consideration the thermal expansion coefficient of water. The differential curves were obtained from the integral ones by calculating the increment of the optical density per 1 °C temperature growth. Thermodynamic parameters (ΔH^0, ΔS^0) were calculated according to Ref. [6].

CD SPECTROSCOPY

The circular dichroism spectra of each complex at various temperatures (from 15° C up to 95 °C) were recorded on J-600 spectropolarimeter (Jasco, Japan). Wavelength range 220–400 nm was used to monitor the changes of the signals in DNA absorption region. The concentration of each component of DNA complexes was 5×10^{-6} M. Temperature control was accomplished by using a jacketed 1 cm cell.

No changes in CD spectra and melting curves were demonstrated in control experiments with single-stranded oligonucleotide target **3**. Differential melting curves of Co(II)-phthalocyanine conjugate **2** have a pronounced transition at 20 °C, indicating that the phthalocyanine moiety interacts with its own oligonucleotide moiety.

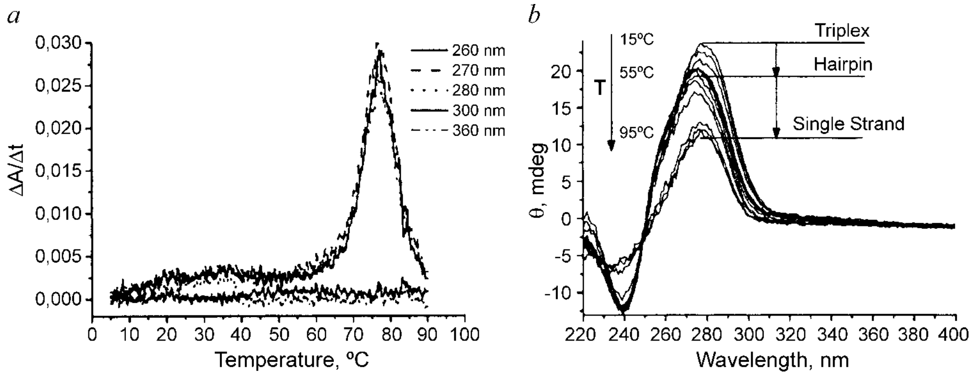

Figure 2. Multiwavelength optical melting curves (a) and temperature changes in the CD spectrum (b) of the complex **8**.

Table 1. Thermodynamic parameters of dissociation of duplexes and triplexes.

Structure	ΔS^{0b}, cal/(K × mol)	ΔH^{0b}, kcal/mol	Tm, ° C	$\Delta G^{0b}{}_{25}$, kcal/mol	$\Delta G^{0}{}_{37}$, kcal/mol
2[a]	135.9	39.5	17.6	− 1.0	− 2.6
4	180.1	61.5	28.1	7.8	5.7
5	156.7	55.6	34.2	8.9	7.0
6[a]	186.2	65.5	76.9	10.0	7.8
7[a]	181.5	63.5	76.4	9.4	7.2
8	70.0	27.6	20.1	6.7	5.9
8[a]	230.5	80.6	76.7	11.9	9.1
9[a]	168.8	58.6	74.0	8.3	6.3
10[a]	179.6	62.3	73.6	8.8	6.6
11	153.3	52.0	19.9	6.3	4.5
11[a]	174.9	60.9	75.0	8.8	6.7

[a] Intermolecular transition.

[b] Estimates of standard errors for ΔS^0, ΔH^0, ΔG^0 are 19, 18 and 11%, respectively.

The differential melting curves and CD signal vs. temperature curves of oligonucleotides **6** and **9** contain only one well-defined maximum at 70–80 °C. This transition corresponds to hairpin dissociation.

On the differential melting curves of duplexes **4** and **5** only one transition was observed. The phthalocyanine residue in duplex **5** led to an increase of $\mathbf{T_m}$ by 7 °C in comparison with duplex **4**.

The possibility of triplex formation **7**, **8**, **10** and **11** was investigated. Differential curves of these structures contain the maximum at 75–80 °C corresponding to hairpin melting. For structures **8** and **11** containing phthalocyanine conjugates one more transition was observed at 20–40 °C. Such a transition is known to be corresponding to triplex dissociation. Typical changes in circular dichroism spectra and melting curves are shown on Fig. 2 for complex **8**. The triplex structures **7** and **10** without phthalocyanine moiety were not detected.

Thermodynamics obtained by UV melting curves are listed in Table 1. It is seen that ΔG^0 value calculated for 25 and 37 °C is increased if a phthalocyanine moiety is present in the duplex (see data for structures **4** and **5**). The values of ΔG^0 are approximately the same within standard error for the hairpin dissociation in structures **6–11**. Formation of triplexes **7** and **10** were not detected for oligonucleotide **1** without phthalocyanine residue, but conjugate **2** is capable to form triplexes **8** and **11** with oligonucleotides **6** and **9**, respectively.

The data presented in this work demonstrate that a phthalocyanine moiety in DNA complexes increases their stability. These results would be useful for the design of antisense oligonucleotide derivatives.

ACKNOWLEDGMENTS

This work was supported by the grant from the President of Russia for support of leading scientific schools, by RFBR (grant No. 02-04-48080), by Russian National

Program on Gene-Directed Biologically Active Compounds, by the Integration grant from the Russian Academy of Sciences (No. 10.6) and by Novosibirsk Regional Foundation for Support of Science and High Education. V. V. K. was supported by grant (No. Y1-B-08-16) from CRDF and from the Russian Ministry of Education within the Program ‘‘Basic Research and High Educaton.’’

REFERENCES

1. Lukyanets, E.A. New sensitizers for photodynamic therapy. Russ. Chem. J. **1998**, *42*, 9–16.
2. Kuznetsova, N.A.; Kaliya, O.L. Photocatalytic generation of active oxygen forms in biological environments in a method of photodynamic therapy. Russ. Chem. J. **1998**, *42*, 36–49.
3. Abramova, T.V.; Ivanova, T.M.; Koval, V.V.; Fedorova, O.S. Synthesis of new oligonucleotide derivatives with porphyrins and phthalocyanins. Nucleosides Nucleotides **1999**, *18*, 1515–1516.
4. Koval, V.V.; Chernonosov, A.A.; Abramova, T.V.; Ivanova, T.M.; Fedorova, O.S.; Derkacheva, V.M.; Lukyanets, E.A. Photosensitized and catalytic oxidation of DNA by metallophthalocyanine-oligonucleotide conjugates. Nucleosides Nucleotides Nucleic Acids **2001**, *20*, 1259–1262.
5. Koval, V.V.; Chernonosov, A.A.; Abramova, T.V.; Ivanova, T.M.; Fedorova, O.S.; Knorre, D.G. The synthesis of a cobalt (II) tetracarboxyphthalocyanine-deoxyribooligonucleotide conjugate as a reagent for the directed DNA modification. Russ. J. Bioorgan. Chem. **2000**, *26*, 104–110.
6. Petersheim, M.; Turner, D.H. Base-stacking and base-pairing contributions to helix stability: thermodynamics of double-helix formation with CCGG, CCGGp, CCGGAp, ACCGGp, CCGGUp, and ACCGGUp. Biochemistry **1983**, *22*, 256–263.

Received December 2, 2003
Accepted April 23, 2004