Vol. 154, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1988 Pages 252-257

POLY(rA) BINDING OF α-ANOMERIC AND ß-ANOMERIC TETRATHYMIDYLATES COVALENTLY LINKED TO AN INTERCALATING OXAZOLOPYRIDOCARBAZOLIUM. DETERMINATION OF THE BINDING PARAMETERS

Claudie Gautier, Didier Bazile and Jacques Paoletti [1]

Laboratoire de Biochimie, INSERM U.140, CNRS LA.147
Institut Gustave-Roussy 94805 Villejuif,France

Received June 2, 1988

We have investigated by means of absorbance measurements at 310 nm the binding of α-anomeric or ß-anomeric tetrathymidylates covalently substituted at their 3′ end by an intercalating agent (oxazolopyridocarbazolium), to poly(rA). Taking into account the strong autoaggregation of the free ligands, we have derived the binding parameters corresponding to the [α] and the [ß] ligands. The affinity of the α-anomer for poly(rA) is higher than the affinity of the ß-anomer in accordance with the Tm studies conducted on such a system.

During the past few years, there have been a growing interest in using oligodeoxynucleotides to control gene expression (see reference 1 for a review). Among these studies, it has been shown that the stability of the annealed complexes formed by oligodeoxynucleotides with their complementary sequence could be strongly enhanced by covalent attachment of an intercalating agent to either end of the oligonucleotide (2-5). More recently, the use of α-anomer of deoxynucleosides, to synthetize such oligonucleotides has improved their stability toward nucleases (6-8). Among these molecules, α-oligodeoxynucleotides modified by means of the linkage of an ellipticine derivative (α-T4C5OPC) were used and appeared to have a greater affinity toward poly(rA) than the corresponding modified ß-oligonucleotide (ß-T4C5OPC)(9). However, the precise determination of their affinity constant was difficult to handle as long as a strong autoaggregation appears for the free compound and is competing with the binding to poly(rA). In order to determine

(1): To whom correspondance should be addressed.
Current address: Unité de Biochimie, Institut Gustave-Roussy Pavillon de Recherche, 94805 Villejuif, France

0006-291X/88 $1.50

accurately the binding parameters of these molecules, we have used a model previously developed by Schwarz and al (10-12). Once taken into account the equilibrium concentrations of free and bound monomer ligands, we have evaluated the basic parameters corresponding to the poly(rA) binding of α- and ß-T4C5OPC respectively. We thus quantitatively confirm that the affinity of α- T4C5OPC for poly(rA) is higher than the one calculated for ß-T4C5OPC in the same conditions.

Materials and Methods

α-T4C5OPC and ß-T4C5OPC were synthetized as previously described (9). The solutions were always freshly prepared before the measurements in order to avoid any degradation of the dissolved solute.

Poly(rA) was purchased as a sodium salt from Boerhinger (Mannheim): Lot 10421221-18.

All the experiments were performed using a cacodylate concentration of 10 mM and a NaCl concentration of 100 mM. The pH was kept equal to 7 and the temperature set at 8°C.

Spectrophotometric measurements were carried out by means of a thermostated Uvikon 810 spectrophotometer equipped with a temperature attachement. During the experiments, nitrogen was circulated through the cuvettes compartment.

Results and Discussion

Aggregation of the free ligand:

The molar extinction coefficients of the T4C5OPC derivatives were found to be dependent on the concentration even in dilute solutions (9). This deviation from the Lambert-Beer's law is due to an aggregation process. this process has already been observed when studying the OPC moiety alone. Assuming that in our conditions of dilution ($C_a^\circ < 10^{-5}$ M) we can neglect the formation of aggregates larger than dimer, we can analyse the optical properties of the T4C5OPC solutions using the procedure previously described by Schwarz et al (12). Let ϵ, ϵ_a and ϵ_d denote the extinction coefficient of the solution, the monomer and the dimer respectively. The total extinction must be:

$$E = C_a^\circ \epsilon = C_a \epsilon_a + 2C_d \epsilon_d \quad [1]$$

C_a°, C_a, C_d being the concentrations of the solution, of the monomer and of the dimer respectively. According to mass conservation, $C_a^\circ = C_a + 2C_d$ [2]. We can then express the fraction of monomer as: $\Gamma_a = C_a/C_a^\circ = (\epsilon - \epsilon_d)/\Delta\epsilon$ [3] were $\Delta\epsilon = \epsilon_a - \epsilon_d$. If K_d represents the equilibrium constant for the aggregation process, we have: $C_d = K_d C_a^2$ [4] and eliminating C_a and C_d between [2], [3] and [4], we derive the following equation:

$$[(\epsilon_a-\epsilon)/C_a^\circ]^{1/2} = (2K_d/\Delta\epsilon)^{1/2}[\Delta\epsilon-(\epsilon_a-\epsilon)] \quad [5]$$

Plotting $[(\epsilon_a-\epsilon)/C_a^\circ]^{1/2}$ versus $(\epsilon_a-\epsilon)$ yields a straight line with the intercepts corresponding to $(2K_d/\Delta\epsilon)^{1/2}$ (ordinate axis) and $\Delta\epsilon$ (on the abcissa axis). Such plot is shown on figure 1 for α-T4C5OPC and ß-T4C5OPC solutions. The molar extinction coefficients of the monomer and the dimer at 318 nm turned out to be: $\epsilon_a(\alpha)$ = 59500, $\epsilon_d(\alpha)$ = 23500 and $\epsilon_a(\beta)$ = 54500, $\epsilon_d(\beta)$ = 26500 for α-T4C5OPC and ß-T4C5OPC respectively. The very good linearity obtained in both cases is in accordance with the assumption that the equilibrium is mainly between monomer and dimer species and that larger aggregates, in our experimental conditions can be neglected. Dimerization constants K_d as well as enthalpy changes ΔH_d (resulting from the temperature dependance of the dimerization) are shown in Table 1. The value of K_d does not strongly differ when comparing α- and ß- anomers: $K_d(\alpha) = 4.5\ 10^5\ M^{-1}$, $K_d(\beta) = 2.2\ 10^6\ M^{-1}$.

Binding of α-T4C5OPC and ß-T4C5OPC to poly(rA):

In the presence of poly(rA), the T4C5OPC solutions exhibit extinction coefficients which differ from those corresponding to the free molecules in solution. As previously observed (9), at a poly(rA) to T4C5OPC ratio: $p = C_p/C_a^\circ = 8$, the ϵ values decreases as the total concentration of drug increases. This decrease corresponds to the spectral changes induced by the binding of the OPC moiety to poly(rA). Assuming n binding sites per unit segment of poly(rA), a fraction Θ of occupied sites and ϵ_b the extinction coefficient of the bound dye at 310 nm, we obtain for ϵ the following equation: $\epsilon = \Gamma_a\epsilon_a+2K_dC_a^\circ\Gamma_a\epsilon_d+\Theta np\epsilon_b$ [6]. This equation takes into account the autoaggregation process of the free dye through Equ.[4]. Furthermore, mass conservation: $\Gamma_a+2K_dC_a^\circ\Gamma_a^2+\Theta np = 1$ [7] allows us to eliminate Θnp from [6] leading to $\epsilon=\epsilon_b+(\epsilon_a-\epsilon_b)\Gamma_a+(\epsilon_d-\epsilon_b)2K_dC_a^\circ\Gamma_a^2$ [8].

From autoaggregation studies, we have access to ϵ_a and ϵ_d and we can calculate K_d (Table 1). By increasing C_a°, keeping p constant, greater and greater fractions of T4C5OPC molecules will bind to poly(rA) and therefore ϵ_b can be determined by extrapolating ϵ to an infinite total concentration of ligand (i.e. $1/C_a^\circ \Rightarrow 0$) as shown in figure 2. Let Γ_a^* represents the total fraction of non bounded ligand (1-Θnp), then Equ [7] becomes: $\Gamma_a^*=\Gamma_a(1+2K_dC_a^\circ\Gamma_a)$ [9] and from the experimental determinations of ϵ, we have access to Γ_a through Equ.[8] and to

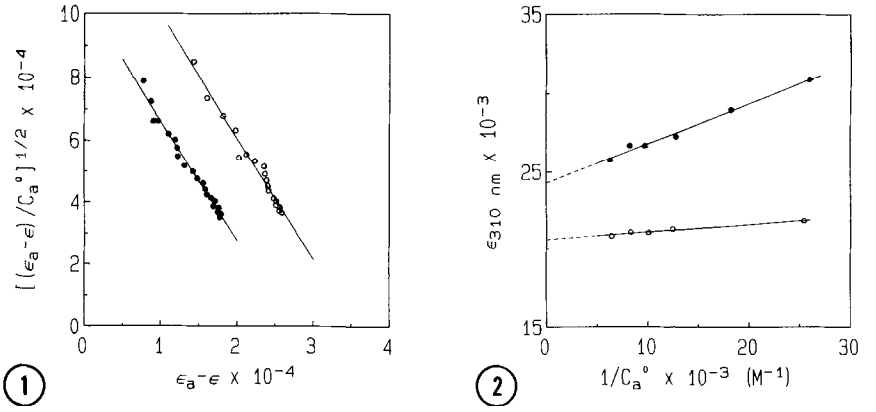

Figure 1: Plots to determine dimerization constants (K_d) and extinction coefficients (ϵ_a, ϵ_d) for ß-T4C5OPC —●— and α-T4C5OPC —○— respectively.Measures were performed at 318 nm.

Figure 2: Molar extinction of ß-T4C5OPC —●— and α-T4C5OPC —○— respectively as a function of the reciprocal value of the total drug concentration ($1/C_a^\circ$) at a constant poly(rA) to T4C5OPC ratio: p=8. The experimental points were measured at 310 nm.

Γ_a^* through Equ.[9]. By plotting Γ_a^* as a function of p with C_a° as parameter (Fig 3) we obtain curves with a common limiting straight line for small p's. This common straight line can be extrapolated to the p axis where its intercept corresponds to np = 1 (p=1/n). This representation leads to values of n which are equal to 0.26 and 0.30 for n(ß) and n(α) respectively. This

Table 1: Extinction and thermodynamic parameters for free T4C5OPC and for T4C5OPC binding to poly(rA) at 8°C

		ß-T4C5OPC	α-T4C5OPC
Free Compound	ϵ_a ($M^{-1}cm^{-1}$)	41800±6200	44000±3500
	ϵ_d ($M^{-1}cm^{-1}$)	32000±4100	27400±2300
	K_d (M^{-1})	$(2.2\pm0.1)\times10^5$	$(4.5\pm0.2)\times10^5$
	ΔH° (kcal/mole)	-8.7	-7.6
	ΔS° (u.e.)	-6.2	-1.4
	ΔG° (kcal/mole)	-7.0	-6.5
Associated to poly(rA)	ϵ_b ($M^{-1}cm^{-1}$)	24300±200	20600±75
	n	0.26±0.004	0.29±0.009
	K (M^{-1})	$(7.0\pm1.0)\times10^5$	$(2.2\pm0.3)\times10^5$
	ΔG° (kcal/mole)	-7.6	-8.3

The thermodynamic parameters for free T4C5OPC are the parameters corresponding to dimerization.The values of ΔG° are given for a temperature of 281°K (8°C)

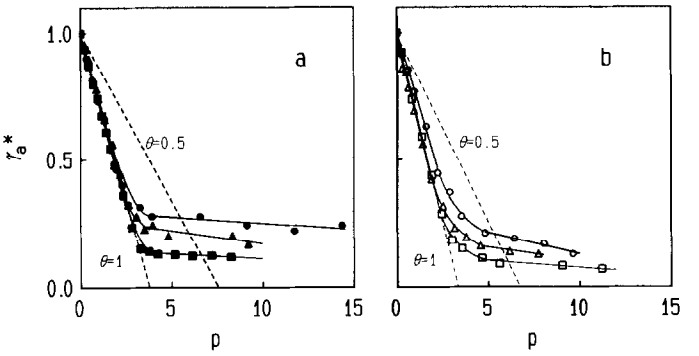

Figure 3: Fraction of free T4C5OPC (τ_a^*) as a function of poly(rA) to drug ratio p.
Panel a refers to ß-T4C5OPC with C_a° = 6.9 µM —●—, 10.3 µM -▲—, and 42.5 µM —■—; panel b refers to α-T4C5OPC with C_a° = 2.9 µM —○—, 5.9 µM —△—, and 8.5 µM —□—. The dashed lines are drawn to show the determination of n and K (see text).

result implies that one molecule of ß-T4C5OPC is bound to 4 adenine residues and corresponds to the binding of one thymine per one adenine residue. In the case of α-T4C5OPC, one molecule of modified oligonucleotide is bound to 3.4 adenine residues. In order to determine the association constants for the binding: K, we define the parameter $s = KC_a = KC_a^\circ\Gamma_a$, and from [9] we derive the following relation:

$\Gamma_a^*=(s/KC_a^\circ)[1+2(K_d/K)s]$ [10]. On figure 3, by drawing a straight line whose equation is $\Gamma_a^*=1-np/2$, we determine an intercept with the experimental curve: $\Gamma_a^*=1-\Theta np$ at a point Γ° where $\Theta=0.5$ i.e. $s=1$ (see ref.11 for discussion). Then by injecting these values of Γ° in Equ 10, K comes out to be the root of the quadratic equation: $K^2C_a^\circ\Gamma^\circ-K-2K_d=0$. Solving this equation for K leads to values of K which are: $K(\alpha)=2.2\ 10^6\ M^{-1}$ and $K(ß)=7\ 10^5\ M^{-1}$ for the binding of α- and ß- T4C5OPC respectively, to poly(rA). As suggested from the melting temperature of the complexes formed between poly(rA) and these modified oligonucleotides (9), the association constant corresponding to the hetero hybrid α-ß is higher than the one corresponding to the ß-ß hybrid. The values ΔG derived from the determination of K (Table 1): $\Delta G^\circ(ß)$ = 7.6 Kcal/mole and $\Delta G^\circ(\alpha)$ = 8.3 Kcal/mole, fit with those derived from the Tm measurements (9). In addition, from these values it appears that the dimerization constants measured for T4C5OPC solutions are of the same order of magnitude than the association constants for

poly(rA). This leads to the conclusion that in such a case, general when studying the interaction of ellipticine derivatives with oligonucleotides, the dimerization process is strong enough to compete with the binding of the drug.

Acknowledgments: This work was supported by INSERM, U.140 and CNRS, LA.147. C.G. was supported by a fellowship CNRS-SANOFI and D.B. by a fellowship from l'Association pour la Recherche sur le Cancer (ARC).

References

1. Green, P.J., Pines, O. and Inouye, M. (1986) Ann.Rev.Biochem. 55, 569-597

2. Asseline, U., Thuong, N.T., and Hélène, C. (1983) C.R.Acad.Sci.Paris 297(3), 369-372

3. Asseline, U., Delarue, M., Lancelot, G., Toulmé, F., Thuong, N.T., Montenay-Garestier, T. and Hélène, C. (1984) Proc. Natl. Acad. Sci. USA 81, 3297-3301.

4. Asseline, U., Toulmé, F., Thuong, N.T., Delarue, M., Montenay-Garestier, T. and Hélène, C. (1984) E.M.B.O. J. 3, 795-800

5. Hélène, C., Montenay-Garestier, T., Saison, T., Takagushi, M., Toulmé, J.J., Asseline, J., Lancelot, G., Maurizot, J.C., Toulmé, F. and Thuong, T. (1985) Biochimie 67, 777-783

6. Morvan. F., Rayner, B., Imbach, J.L., Chang, D.K. and Lown, J.W. (1986) Nucl. Acids Res. 14, 5019-5035

7. Morvan, F., Rayner, B., Imbach, J.L., Thenet, S., Bertrand, J.R;, Paoletti, J., Malvy, C. and Paoletti, C. (1987) Nucl. Acids Res. 15, 3421-3437

8. Sun, J.S., Asseline, U., Rouzaud, D., Montenay-Garestier, T., Thuong, N.T. and Hélène, C. (1987) Nucl. Acids Res. 15, 6149-6158

9. Gautier, C., Morvan, F., Rayner, B., Huyn-Dinh, T., Igolen, J., Imbach, J.L., Paoletti, C. and Paoletti, J. (1987) Nucl. Acids Res. 15, 6625-6641

10. Scharwz, G. (1968) Biopolymers 6, 873-897

11. Schwarz, G. (1970) Eur.J.Biochem. 12, 442-453

12. Schwarz, G., Klose, S. and Balthasar, W. (1970) Eur.J.Biochem. 12, 454-460