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Hybridization of a phosphate-methylated adenosine trinucleotide with natural oligothymidines of various lengths. A thermodynamic consideration

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SUMMARY

Stability data are presented for the hybrids between phosphate-methylated $d(acA_3ac)$ and the natural strands poly(dT), $d(T_{30})$, $d(T_{10})$, and $d(T_5)$. Hybridization could be established for poly(dT) and $d(T_{30})$, showing melting transitions (T_m) of 41°C and 27°C, respectively. This suggests a cooperative behaviour of the phosphate-methylated trimers in duplex formation. A thermodynamic model is presented which adequately describes the experimental data. On the basis of this model, it is expected that longer phosphate-methylated systems will be able to hybridize effectively with complementary natural DNA sequences.

INTRODUCTION

Recently, we reported that the phosphate-methylated, neutral trimer $d(acA_3ac)$ forms a hybrid with the complementary natural poly(dT), which has a melting temperature (T_m value) of 41°C (Koole et al., 1987a). Under identical experimental conditions, the hybrid of natural $d(A_3)$ and poly(dT) dissociates at 20°C. This difference in duplex stability is caused by the elimination of interstrand electrostatic repulsions between the phosphate groups after methylation. In addition, we established that phosphate-methylated $d(acA_2ac)$ and $d(acA_4ac)$ also form hybrids with natural poly(dT), with T_m values of 30°C and 57°C, respectively (van Genderen et al., 1987a). We now report new data on the duplex stability of hybrids between phosphate-methylated $d(acA_3ac)$ and natural $d(T_k)$ oligomers, which show that the length of the oligothymidine strand also influences the T_m value of the hybrid. A thermodynamic model is presented which describes the observed effects adequately.

RESULTS AND DISCUSSION

T_m values were determined with UV hyperchromicity experiments for the hybrids of phosphate-methylated $d(acA_3ac)$ and poly(dT), $d(T_{30})$, $d(T_{10})$, and $d(T_5)$. In the case of $d(acA_3ac)$ and poly(dT), the T_m value was measured independently with 500 MHz 1H NMR. With $d(T_5)$ and $d(T_{10})$ no duplex \rightleftharpoons coil transition above $15^\circ C$ is found, whereas with $d(T_{30})$ and poly(dT) the hybrids exhibited T_m values of respectively $27^\circ C$ and $41^\circ C$ (vide supra). In all systems, phosphate-methylated $d(acA_3ac)$ was added in such a ratio, that all thymine bases could form A – T base pairs. When an adenine to thymine ratio of 1:2 was used, the T_m values were lowered to $39.5^\circ C$ for phosphate-methylated $d(acA_3ac)$ with poly(dT), and to $23^\circ C$ with $d(T_{30})$. The stability data show that the hybridization of phosphate-methylated $d(acA_3ac)$ with a $d(T_k)$ strand is a *cooperative* process, i.e., each attached molecule facilitates hybridization for the next one. It should be noted, that hybridization between short fragments of phosphate-methylated DNA has been observed, even for phosphate-methylated $d(T_2)$ (Koole et al., 1987b). Also, duplexes of short pieces of natural DNA have been reported (Young & Krugh, 1975). These observations show that the conformations of both phosphate-methylated and natural DNA are correct for duplex formation. However, when the conformation of the phosphate-methylated DNA does not fit exactly with that of natural DNA, then the natural DNA must adopt a new conformation. For short strands with a random conformation, this will incur a significant loss of entropy. Larger strands of e.g. thymidines already possess a single-helix geometry (Camerman et al., 1976), and can undergo a conformational change without a loss of entropy. After hybridization of one phosphate-methylated molecule, the second molecule can hybridize more easily since the natural DNA has then partially adapted its conformation for hybridization. This cooperative process can be expressed in a thermodynamic model.

THERMODYNAMIC MODEL

The process of duplex formation itself between one phosphate-methylated $d(acA_nac)$ and a $d(T_k)$ strand involves a change in enthalpy and entropy (ΔH_n and ΔS_n , if one assumes $n < k$). The necessary change in conformation of the $d(T_k)$ strand gives another entropy change ($\Delta S'_k$). Then the T_m value can be expressed as:

$$T_m = \frac{\Delta H_n}{\Delta S_n + \Delta S'_k}$$

which is a function of both n and k . Association of a second phosphate-methylated $d(acA_nac)$ molecule involves the same ΔH_n and ΔS_n terms, but the $d(T_k)$ strand has at least partially adopted the correct conformation for hybridization. Therefore only a term $\alpha \Delta S'_k$ is involved, with $\alpha \in (0,1)$. The parameter α is a measure of the cooperativity, ranging from no effect ($\alpha = 1$) to a complete conformational change after the first hybridization ($\alpha = 0$). In our

experiments, α most likely has an intermediate value. Then, a rise in T_m value is seen, compared to hybridization of one molecule:

$$T_m = \frac{\Delta H_n}{\Delta S_n + \frac{1+\alpha}{2} \Delta S'_k} > \frac{\Delta H_n}{\Delta S_n + \Delta S'_k}$$

If one assumes that each next hybridization involves another factor α , association of p molecules of phosphate-methylated $d(acA_nac)$ with a $d(T_k)$ strand results in a generalized expression for T_m :

$$T_m = \frac{\Delta H_n}{\Delta S_n + \frac{1-p^{-1}}{p} \sum_{i=0}^{p-1} \alpha^i \Delta S'_k}$$

This relation can also be written as:

$$T_m = \frac{\Delta H_n}{\Delta S_n + \frac{\Delta S'_k}{p} \cdot \frac{1-\alpha^p}{1-\alpha}}$$

For long fragments of natural DNA (large k), $\Delta S'_k$ becomes very small, and $T_m \approx \Delta H_n / \Delta S_n$, which is only a function of the length of the phosphate-methylated DNA. For short fragments of natural DNA (small k), $\Delta S'_k$ is a large (negative) quantity, so T_m will be lower, despite the same length of the phosphate-methylated DNA.

Based on the present experimental data of the hybrids of phosphate-methylated $d(acA_3ac)$ with poly(dT) and $d(T_{30})$, the values of $\Delta S'_k$ for $k = 300$ (average length of poly(dT)) and $k = 30$ can be calculated, as well as ΔS_3 and α , the cooperativity parameter. As we have determined earlier, ΔH_3 equals -23.4 kcal/mol (Kooles et al., 1987a). Solving the equations for the four T_m values reported above then yields: $\Delta S_3 = -74.1$ cal/mol \cdot K, $\Delta S'_{30} = -6.2$ cal/mol \cdot K, $\Delta S'_{300} = -4.4$ cal/mol \cdot K, and $\alpha = 0.89$.

Indeed, the loss of entropy $\Delta S'_k$ depends on the value of k , and is smaller for longer fragments of natural DNA. The cooperative effect is seen in the value of α , which is indeed smaller than unity. Further experiments will lead to a more detailed model description for these hybridization processes.

CONCLUDING REMARKS

From this model, it can be understood why the T_m value of a hybrid between phosphate-methylated and natural DNA is related to the length of both strands, and reaches a limiting value for a large natural DNA. The effect is based on the rigid conformation of the phosphate-methylated DNA, which does not fit exactly with that of natural DNA. A similar effect has been seen in our recent work on the hybridization between phosphate-methylated $d(acA_3ac)$ and the natural RNA poly(U) (van Genderen et al., 1987b). No duplex formation occurred, which implies that the phosphate-methylated DNA

can not adopt the A geometry necessary for DNA/RNA association. The RNA molecule is also rigid, and does not adapt itself. A DNA single strand can change its conformation, and hence duplex formation is seen between short phosphate-methylated oligomers and long natural DNA. Increase of the length of the phosphate-methylated DNA can compensate for a smaller length of the natural DNA, so we expect that $d(T_{30})$ is no lower limit for hybridization at room temperature. Long phosphate-methylated oligomers are expected to hybridize strongly with complementary single-stranded DNA, thus giving the possibility for highly specific blocking of a part of the natural DNA.

MATERIALS AND METHODS

UV experiments were performed on a Perkin Elmer 124 spectrophotometer at a wavelength of 260 nm. The oligomers $d(T_5)$, $d(T_{10})$, and $d(T_{30})$ were synthesized on an Applied Biosystems 381 A DNA synthesizer. Phosphate-methylated $d(acA_3ac)$ was prepared as described earlier (Koole et al., 1987a).

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