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Thermodynamics and mechanism of ssDNA hybridization below the melting temperature by isothermal titration calorimetry

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Abstract

This investigation presents measurements of isothermal ssDNA oligomer hybridization enthalpy at various DNA length, GC contents, temperatures, and salt concentrations by isothermal titration calorimetry (ITC) at temperatures below the melting temperature of the DNA. The study aimed to reveal the hybridization mechanism of ssDNA for gene chip applications. The role of hydrogen bonds between complementary bases, the stacking forces between bases, the electrostatic repulsion between strands, the dehydration and conformational rearrangement of ssDNA in hybridization are estimated from the interaction enthalpy.

Keywords: DNA hybridization; Enthalpy; Isothermal titration calorimetry; Gene chip

1. Introduction

The development and application of DNA biochips have attracted interest in understanding the mechanism of DNA hybridization. It is generally believed that hydrogen bonding, π-stacking between the base pairs of complementary strands, and the conformational change of single-strand DNA (ssDNA) play major roles in the formation of double-strand DNA (dsDNA). The thermodynamics of hybridization provides evidence of the forces and mechanisms involved in DNA–DNA interactions. However, the hybridization enthalpies have not been well documented, particularly at temperatures significantly lower than the melting temperature (T_m) of dsDNA, temperatures common in gene chip applications.

The enthalpy–entropy compensation relationship has been observed in various ssDNA dinucleotides hybridization with and without mismatch at temperatures below T_m [1]. The results indicate that weak intermolecular forces between ssDNA are important in hybridization. Water molecules are essential to stabilize the secondary and tertiary structures of biomolecules, and the hydration states of the DNA result in various forms of DNA. Therefore, in the hybridization of two ssDNAs, desolvation (removal of structural or electrostricted water molecules [2] and counter ions) of ssDNA and the destruction of intra-ssDNA stacking and hydrogen bonding (conformational rearrangements) are essential before hybridization can occur. These processes require energy but contribute entropy partially owing to the release of water in the desolvation processes. The change in heat capacity accompanying duplex formation from ssDNA [3] suggests an apparent conformational change of ssDNA from the unstructured single stranded state to the helical (stacked) single stranded state [4]. Additionally, the energy increase required by conformational change at a higher temperature indicated that hydrogen bonding and π-stacking intramolecular forces are important in ssDNA hybridization [5].

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The melting temperature (T_m) and enthalpy of dsDNA have been measured by differential scanning calorimetry (DSC) [6]. However, reported enthalpies are not applicable in gene chips, which are normally used at a temperature below T_m. Furthermore, the melting enthalpy measured by DSC is a function of temperature.

The hybridization enthalpy applicable to gene chip applications is the enthalpy change between the initial state (two ssDNAs at the environment temperature) and the final state (hybridized dsDNA at the environment temperature). The enthalpy change for ssDNA hybridization in gene chips should thus be investigated at constant temperature via isothermal titration calorimetry (ITC).

ITC provides thermodynamics information for various biomolecular interactions [7] and proteins with solid surface [8]. ITC has been used to study ssDNA hybridization [9], but the highly exothermic dilution heat of highly charged ssDNA in solution may not have been accurately presented. Therefore, the dilution heat of both ssDNAs (target and probe) in buffer must be precisely estimated.

The present study employed ITC to measure the hybridization enthalpy between ssDNAs of various lengths and GC content at different temperatures and salt concentrations to assess the hybridization enthalpy and mechanism at the temperatures below the T_m of dsDNA. These results will benefit developers and end-users in designing and choosing gene chips.

2. Materials and methods

2.1. Materials

Deoxyribonucleic acids (DNA) were obtained from Sinopharm (Taiwan) and are listed in Table 1. Tri-sodium citrate dihydrate was obtained from Merck (Germany). Sodium chloride and sodium dodecyl sulfate (SDS) were all obtained from Sigma (USA). The SSC buffer was prepared by mixing 3 M sodium chloride with 0.3 M tri sodium citrate dihydrate and defined as the 20× SSC buffer. The 2× SSC and 5× SSC buffers were diluted directly from the 20× SSC.

2.2. Methods

Isothermal titration calorimetry was done with a thermal activity monitor (TAM) (Thermometric AB, Sweden), with a 4 ml stainless-steel ampoule filled with a solution of target ssDNA. When thermal equilibrium was reached between the ampoule and the heat sink, the probe ssDNA solution was titrated into the ampoule via a Hamilton syringe fitted with a stainless-steel needle driven by a computer-controlled pump. The output power, P, was integrated with time and divided by the quantity of ssDNA titrated. The apparent heat from titration was corrected by subtracting the dilution heat of both the target and probe ssDNA solutions to obtain the net heat of interaction between ssDNAs. The dilution heat of the probe ssDNA was obtained by injection the same amount of target ssDNA as for the measurement of hybridization into the ampoule filled with hybridization buffer without target ssDNA, while the dilution heat of target ssDNA was done by titrating buffer into the ampoule with target ssDNA. The hybridization enthalpy (ΔH) thus can be calculated using the following equation [8]:

$$Q = Vq^* \Delta H$$

Q (kJ) denotes the net heat of the interaction between ssDNAs after the subtraction of both dilution heat of target and probe ssDNA. V (ml) represents the volume of ssDNA solution in

Table 1
Sequence and notation of the single-strand DNA oligomers used in this investigation

	5′-DNA sequence-3′	Length	MW (g mol^{-1})	T_m (°C)	GC/(mol.%)
ST5	AAGGG	5-mer	1552	16	60
SP5	CCCTT	5-mer	1414	16	60
$ST7_1$	GGGAAAA	7-mer	2179	20	42.9
$SP7_1$	TTTTCCC	7-mer	2022	20	42.9
$ST7_2$	AAAGGGG	7-mer	2195	22	57.1
$SP7_2$	CCCCTTT	7-mer	2007	22	57.1
$SP15_1$	CATCCGTGTGGTAAC	15-mer	4554	46	53
$ST15_1$	GTTACCACACGGATG	15-mer	4563	46	53
$SP15_2$	CGACGACTGGCATGC	15-mer	4564	50	67
$ST15_2$	GCATGCCAGTCGTCG	15-mer	4555	50	67
ST16	GTTAGGACACGGATTG	16-mer	4946	43	50
SP16	CAATCCGTGTCCTAAC	16-mer	4786	43	50
ST17	GTTAGGACGACGGATTG	17-mer	5274	47	53
SP17	CAATCCGTCGTCCTAAC	17-mer	5074	47	53
$ST18_1$	GTTAGGACGACGGGATTG	18-mer	5603	60	56
$SP18_1$	CAATCCCGTCGTCCTAAC	18-mer	5363	60	56
$ST18_2$	GTTATTACAACATTATTG	18-mer	5471	37	22
$SP18_2$	CAATAATGTTGTAATAAC	18-mer	5489	37	22
ST22	GACGTCATCCCCACCTTCCTCC	22-mer	6506	72	63
SP22	GGAGGAAGGTGGGGATGACGTC	22-mer	6346	72	63

S denotes single-strand and T and P represent the target and probe, respectively. The numbers following SP or ST indicate the length of the ssDNA (number of mers).

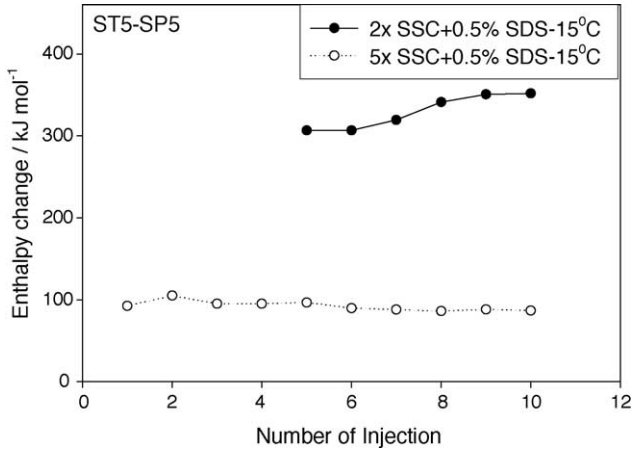

Fig. 1. Variation of the hybridization enthalpy (kJ/mol) of ssDNAs (ST5 and SP5) with ssDNA concentration at 15 °C in 2× SSC buffer with 0.5% SDS.

the ampoule. q^* (mol/ml) is the amount of ssDNA interaction. Stoichiometric hybridization of injected ssDNA with its complementary ssDNA chain in the ampoule is assumed, especially for the temperatures below the melting temperature of the dsDNAs. Each injection volume contained one-tenth of the number of moles of target ssDNA in the ampoule and 10 injections were conducted for the enthalpy measurement; therefore, at the end of the last injection, a 1 to 1 molar ratio was reached between the target and probe ssDNA. The detailed operation of the ITC is described in a previous report [8].

3. Results

The effects of temperature and salt concentration on hybridization enthalpy were investigated first. Experimental temperatures were 15, 25 or 30 °C, which are all lower than the T_m of the dsDNA. Results are illustrated in Figs. 1–3. At

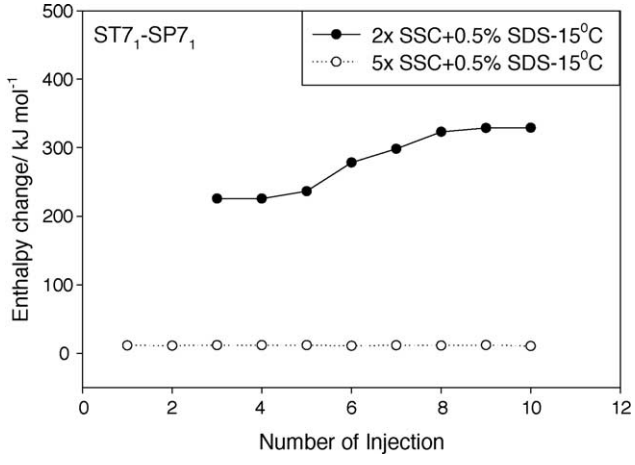

Fig. 2. Variation of the hybridization enthalpy of ssDNAs ($ST7_1$ and $SP7_1$) with ssDNA concentration at 15 °C at 2×, and 5× SSC buffer with 0.5% SDS.

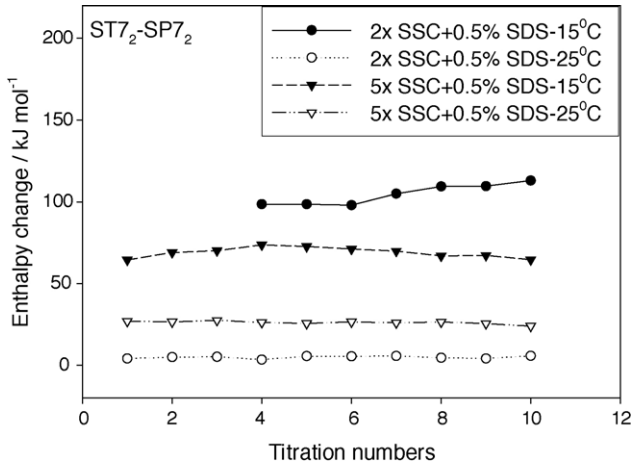

Fig. 3. Variation of the hybridization enthalpy of ssDNAs ($ST7_2$ and $SP7_2$) with ssDNA concentration at 15 and 25 °C, at 2× and 5× SSC buffer with 0.5% SDS.

15 °C, the hybridization enthalpy of 5-mer and 7-mer DNA with their complementary ssDNA in 2× SSC/0.5% SDS or 5× SSC/0.5% SDS were all endothermic and less endothermic is obtained at the hybridization buffer with higher salt concentration. Also, the enthalpy is varied and is more endothermic as the amount of hybridization increase for buffer with low salt concentration, while the enthalpy is almost constant at the buffer with high salt concentration. Fig. 3 also shows the hybridization enthalpies of complementary 7-mer ssDNA hybridization at two different temperatures. The reaction is less endothermic at higher temperature.

This work studied the effects of length of ssDNA (15-mer to 18-mer) and the GC content (GC%). Specifically, ssDNA of 15-mers ($ST15_1$–$SP15_1$; $ST15_2$–$SP15_2$) and 18-mers ($ST18_1$–$SP18_1$; $ST18_2$–$SP18_2$) with designated GC content were selected for this investigation as listed in Table 1. The results were listed in Table 2. Based on the data, three points can be concluded. First at all, more endothermic hybridization enthalpies were observed at increasing salt concentration at 25 °C as demonstrated in Figs. 1–3. Secondly, ssDNA with similar GC content, less endothermic reaction enthalpies were revealed as the ssDNA length increase. Thirdly, higher endothermic enthalpies at low GC% of ssDNA were demonstrated in this investigation.

4. Discussions

The data presented in Figs. 1–3 seem to contradict the exothermic characteristics of forming hydrogen bonding and stacking interaction between the base pairs of complement ssDNAs in hybridization. Examining the hybridization process, however, four distinct subprocesses of the hybridization can be distinguished: intramolecular conformational re-arrangement of ssDNA; dehydration or dissolution of ssDNA; base pairing and then hydration (duplex forming); finally dissolution and accompanying

Table 2
List of the hybridization enthalpies (kJ/mol) of the ssDNAs of 15–22 oligomer in various GC%, temperatures and salt concentrations

DNA pair	GC%	Hybridization enthalpy (kJ mol^{-1})			
		2× SSC + 0.5% SDS		5× SSC + 0.5% SDS	
		25 °C	30 °C	25 °C	30 °C
$ST15_1$–$SP15_1$	53	16.43	10.22	40.77	10.69
$ST15_2$–$SP15_2$	67	4.45	–	27.76	–
ST16–SP16	50	1.76	5.76	19.62	5.17
ST17–SP17	53	7.42	9.27	26.37	7.46
$ST18_1$–$SP18_1$	56	1.17	10.13	12.41	5.47
$ST18_2$–$SP18_2$	22	–	–	64.94	–
ST22–SP22	64	0.96	8.46	−3.05	2.86

structural re-arrangement of dsDNA. The conformational rearrangements of a structured ssDNA, such as hairpin or slipped duplex, as well as the dehydration, require energy and are endothermic subprocesses. In the subprocess of hybridization, from an energy perspective, we should consider that energy is required to overcome the electrostatic repulsion between the highly negatively charged ssDNA and the energy is released from the formation of the hydrogen bonding and π-stacking. Similar discussions have been documented on protein interactions in general molecular recognition processes [8]. Therefore, the endothermic behavior of the hybridization can be contributed to the conformational rearrangement and dehydration subprocess as well as to the repulsive force generated between two ssDNAs.

Theoretical modeling forecasts that 14 water molecules are bound around the A and G, while 12 water molecules are bound around T and C. Moreover, the calculated dehydration enthalpy was 64.67 and 50.03 KJ/mol for [A, G] and [T, C], respectively [10,11]. The enthalpy required for the dehydration dominated the short ssDNA hybrization, and, as a whole, the negligible energy required for the conformational changes of short ssDNA oligomer and the lesser energy released from the formation of hydrogen bonds between the base pair justified the endothermic hybridization process in Figs. 1–3. The enthalpy can be compensated by the entropy gain from the bound water molecules.

The data of lower (2× SSC) salt concentration as shown in Figs. 1–3 implies notably that higher endothermic process was observed with the increasing concentration of dsDNA formed or the increasing number of titration of ssDNA into the ampoule. This resulted can be explained as more energy is needed for dissolution as the solution charge density is higher. This phenomenon was observed only in low salt concentration, demonstrating the importance of electrostatic repulsion in the hybridization. For most of the hybridization involving longer complementary ssDNA, the enthalpy change is essentially not a function of moles of ssDNA titrated. The hybridization enthalpies for longer ssDNA were listed in Table 2 for the following discussions.

In comparison of the enthalpies of the various ssDNA lengths at the two salt concentrations, more endothermic reaction occurred in the 5× SSC condition, indicating that more energy is required for the dehydration process when the DNA molecule solution has higher surface tension.

Moreover, as for the temperature effects at 5× SSC, higher temperature (30 °C) reduced the energy needed for dehydration. It means that less energy is required to break the hydrogen bonds between the bound water with ssDNAs, resulting in a less endothermic process of hybridization at higher temperature conditions. The results were consistent with the reported data [9,12]. Furthermore, for hybridization at 30 °C in 2× SSC and 5× SSC, the effects of the salt concentrations studied were insignificant [9]. Overall, the salt concentration reduces the electrostatic repulsion between two ssDNAs, thus increasing the surface tension of DNA in aqueous solutions and promoting the energy required for dehydration of the bound water. Consequently, from the thermodynamics point of view, within certain ranges of the hybridization conditions, the salt affects more acutely in entropy than in enthalpy.

For the effects of GC content (GC%), the results in Table 2 clearly demonstrate that the released binding energy increased (less endothermic) as more GC pairing occurred. Comparing with ssDNA of different length but similar GC%, the energy generated increased as more bonds forming for ssDNA hybridization. Further calculation of the enthalpy change with GC% difference revealed 1.0–1.5 KJ/mol decreases as we increased GC content. The obtained energy is lower than but still compatible with the reported 2.38 KJ/mol data by Schwarz et al. [13] of 10-mer DNA hybridization. The data in Table 2 also shows that more endothermic hybridization enthalpies were observed at increasing salt concentration at 25 °C as demonstrated in Figs. 1–3. Furthermore, the hybridization enthalpy is affected in less extent by DNA length at higher temperatures and lower salt concentrations suggesting that the dehydration subprocess play profound role among the subprocesses in the view points of thermodynamics.

In Table 2, we shown that, various lengths of full complementary ssDNA hybridization create distinct enthalpies, indicating possible applications of measuring hybridization enthalpy under certain conditions to discriminate the hybridization length (numbers of match pairs). This finding maybe promisingly applied in SNP detection. Nevertheless, the data and arguments in this work are confined in the materials studied and the ranges of temperatures. Higher

temperature would destabilize dsDNA and the hybridization enthalpy may become exothermic near T_m.

5. Conclusions

Our results demonstrate that isothermal ssDNA hybridization at temperature lower than T_m is endothermic and is attributed to the dehydration and structural re-arrangement of ssDNA. The enthalpy is compensated by the entropy gained from the dehydration process of ssDNA. The data presented in this investigation provide basic thermodynamics information for gene chip developer and user.

The hybridization processes in this study were performed isothermally, so that the heat capacity change of the molecules can be neglected. For hybridization performed.

We also examined the endothermic processes of various ssDNA lengths under different hybridization conditions. Similar results (shown in Figs. 1–3) illustrated the effects of salt concentration on DNA hybridization of various ssDNA lengths. Less endothermic hybridization enthalpies were derived under increased salt concentration (2× SSC to 5 or 7× SSC) owing to that the electrostatic repulsive force was repressed. Fig. 3 shows the hybridization enthalpies of complementary 7-mer ssDNA hybridization at two different temperatures. The dehydration subprocess requires less energy at higher reaction temperature.

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