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DNA Denaturation Under Freezing in Alkaline Medium

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Abstract

It is generally accepted that DNA conserves its secondary structure after a freeze-thaw cycle. A negligible amount of degradation occurs after this procedure. Degradation becomes appreciable only after multiple cycles of freezing and thawing. In this study, we have found that a single freeze-thaw cycle in alkaline medium (pH ≥ 10.8) gives rise to denaturation of calf thymus DNA, although the melting temperature of intact DNA in the solution used for the freeze-thaw experiments is higher than 60 °C. The degree of denaturation is almost independent of the regime of freezing. The melting curve obtained after DNA is frozen at -2 °C and then thawed is almost the same as after a freezing carried out in liquid nitrogen (-196 °C). However, incubation in the same solution at 0 °C for 24 hours without freezing does not give rise to any denaturation. The degree of denaturation caused by freezing increases with pH (if pH ≥ 10.8) and decreases with Na_2CO_3 concentration at fixed pH and [Na^+], although Na_2CO_3 decreases the melting temperature of intact DNA. A preliminary treatment of DNA with cisplatin or transplatin (0.01 Pt atoms per nucleotide) gives rise to a full recovery of the DNA secondary structure after freezing and thawing similar to what occurs after heating DNA to 100 °C and quick cooling. Possible mechanisms that may cause DNA denaturation during a freeze-thaw cycle in alkaline medium are discussed.

Introduction

There are two ways that low temperatures can influence a solution of a biopolymer. First, decreasing the temperature of a biopolymer solution without freezing may induce a "cold denaturation" transition of protein (1-5) or RNA (6). Second, freezing of water that accompanies cooling can also change the structure of a protein (7, 8) or RNA (9).

As was shown long ago, multiple freeze-thaw cycles give rise to DNA degradation without denaturation (10-14). Degradation is generally negligible after a single freeze-thaw cycle even if samples are frozen in liquid nitrogen (-196 °C). However, after 38 cycles, there is a threefold decrease in the molecular weight of the phage T2 DNA (11). DNA breaks are thought to arise at cracks of the ice, which are formed during freezing.

In this study, we have found that freezing and thawing of a calf thymus DNA solution in alkaline medium (pH ≥ 10.8) give rise to denaturation, even though the DNA's melting temperature is higher than 60 °C in the solutions used for these studies. The degree of denaturation increased with pH and decreased with the buffer capacity of the DNA solution. The structure of DNA after freezing was similar to that obtained after heating at 95 or 100 °C (Figure 1, denatured DNA-1). Subsequent thawing gives rise to the structure formed by the heating followed by quick cooling (Figure 1, denatured DNA-2). Cooling prevents renaturation (strand association).

It is known that cisplatin binding to DNA makes heat denaturation reversible (15) due to the formation of interstrand crosslinks that account for 6% of cisplatin ad-

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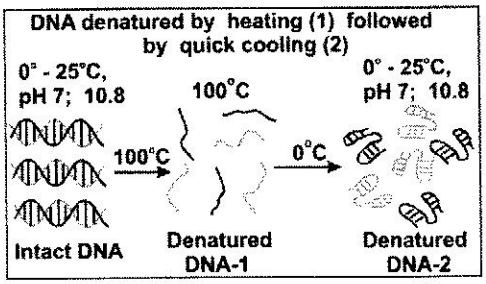

Figure 1: DNA denaturation caused by heating (1) followed by quick cooling (2).

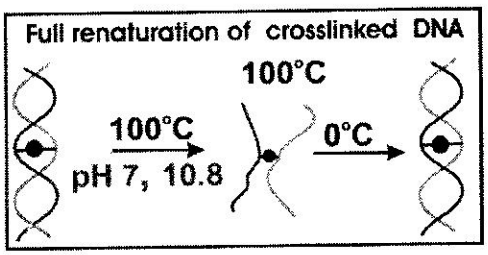

Figure 2: Recovery of the double helix after full melting for DNA with interstrand crosslinks.

ducts (16, 17) (Figure 2). This was demonstrated for neutral medium (15). In this work, we have demonstrated the same full recovery after denaturation caused by heating-cooling in alkaline medium or freezing-thawing in the same medium for DNA preliminarily treated with cisplatin or transplatin at $r \geq 0.01$ (r is the number of Pt atoms per nucleotide).

Materials and Methods

Ultra pure calf thymus DNA that we prepared was used (protein < 0.1%, RNA < 0.1%, molecular mass ~30 MDa). The properties of this DNA have been previously described (18). For interstrand crosslinking, DNA at a concentration of 1.2 mg/ml was incubated with cisplatin or transplatin (Sigma Chemical Company) in 10 mM $NaClO_4$ (pH 6.5). Pt/nucleotide molar ratios (r) were 0.001, 0.01, or 0.05. Cisplatin was added to DNA from a stock solution in distilled water (1 mg/ml) after a long time of incubation at room temperature. The mixture was incubated for 48 hours at 37 °C in the dark. It was demonstrated that under these conditions cisplatin reacted with DNA quantitatively (19-21). Platination was stopped by addition of 0.1M NaCl and freezing at -28 °C (22-24). Platinated DNA can be kept at this temperature up to 6 months without a change in melting properties.

Intact DNA and platinated DNA were diluted to 0.04 mg/ml for denaturation experiments by heating or freezing and further melting experiments. Heat denaturation was carried out in 0.1 M NaCl, $5 \cdot 10^{-5}$ M EDTA, 1mM Na_2CO_3 at pH 7 or 10.8 by a 10-minute incubation at 95 or 100 °C (Figure 1, denatured DNA-1). pH was adjusted by addition of NaOH or HCl and measured at 25 °C. The incubation was followed by quick cooling in ice (Figure 1, denatured DNA-2). After cooling, melting curves of the DNA were measured immediately. Here and below the concentration of Na_2CO_3 added to the DNA solution is given. Depending on pH, the anion CO_3^{-2} partially transforms into HCO_3^- and H_2CO_3. The dependence of the relative concentrations of the three components on pH was described earlier (25).

Freezing of DNA was carried out at -2 °C, -18 °C, and -196 °C in 0.1 M NaCl, $5 \cdot 10^{-5}$ M EDTA at various pH (10.5 to 11.5) and concentrations of Na_2CO_3 (0, 10^{-3}, $5 \cdot 10^{-3}$ M). The time of incubation after freezing was varied from 5 minutes to 48 hours. Thawing was carried out at 25 °C just before melting. Melting studies were carried out in the solutions used for freezing. Freezing and melting of platinated DNA was carried out in 0.1 M NaCl, $5 \cdot 10^{-5}$ M EDTA, pH 10.8 (without Na_2CO_3).

DNA melting curves were determined by measuring the optical density (D) at 260 nm as a function of temperature (T) using a SF-26 spectrophotometer (LOMO, Russia). DNA denaturation after freezing and thawing, or after heating at 95 °C or 100 °C and quick cooling was characterized using the equation $\theta(T)=\Delta D(T)/D$ (18) where $\Delta D(T)=D(T)-D$ (18) is an increase in optical density of DNA solution at the temperature T with respect to the optical density of DNA solution at T=18 °C.

Results

Melting curves of DNA were determined from optical density vs. temperature profiles and represented by $\theta(T)=\Delta D(T)/D$ (18). Curve 1 in Figure 3 was obtained

immediately after dilution of intact DNA in alkaline solution used for freezing (0.1 M NaCl, $5 \cdot 10^{-5}$ M EDTA, 1mM M Na_2CO_3, pH 10.8) and curve 2 was obtained after a 24 hour incubation at 25 °C. The same melting curve was obtained after incubation for 24 hours at 0 °C (without freezing). Comparison of curves 1 and 2 demonstrates that the solution used for freezing-thawing experiments does not damage DNA incubated for 24 hours at 0 °C and 25 °C. The slight stabilization revealed after incubation (compare curves 1 and 2) is caused by a small decrease in pH during incubation.

DNA distortion after heat denaturation is reflected by the appearance of the low temperature part of the melting curve and a decrease in the saturation value of the function $\theta(T)=\Delta D(T)/D$ (18) reached at high temperature when DNA is fully melted (θ_{max}). DNA was denatured in the solution used for freezing or in the same solution adjusted to pH 7 instead of pH 10.8. To produce control melting curves for various degrees of denaturation, calf thymus DNA at pH 7 and pH 10.8 was heated at 95 and 100 °C for 10 minutes and then quickly cooled in ice. Then melting curves were recorded in the solution used for freezing.

If no previous denaturation occurred, θ_{max} of a DNA sample is from 0.37 to 0.39 (Figure 3, curves 1 and 2). For all denatured samples (curves 3-6), a low temperature increase in $\theta(T)$ appears between 18 and 60 °C. Heating a sample at 95 °C (pH 7) results in a denatured DNA with θ_{max}=0.18 (curve 3). Denaturation increases at pH=10.8 (θ_{max}=0.12, curve 4). A further decrease in θ_{max} is observed when DNA is heated at 100 °C (θ_{max}=0.1 and 0.08 for samples at pH 7 and 10.8 respectively, curves 5, 6).

The degree of denaturation (θ_{den}) after freeze-thaw procedure for a given DNA solution was evaluated using Eq.[1]:

$$\theta_{den} = \frac{\theta_i - \theta_{max}}{\theta_i - \theta_d} \qquad [1]$$

where θ_{max} is measured after freezing and thawing; θ_i and θ_d are respectively θ_{max} of intact DNA and DNA heated at 100 °C (and quickly cooled) in the same solution.

Thus, extended incubation at 0° C or 25° C in 0.1 M NaCl, $5 \cdot 10^{-5}$ M EDTA, 1mM Na_2CO_3, pH 10.8 does not give rise to denaturation ($\theta_{den} = 0$) (Figure 3, curve 2 and Figure 4, curve 1). However, freezing DNA in the same solution at temperatures of -2° C, -18° C, or -196° C causes DNA denaturation. Melting curves that demonstrate DNA denaturation after freezing and thawing at these temperatures are represented by curve 3 in Figure 4. After freezing and thawing, $\theta_{max} \approx 0.2$ about two times lower than before this procedure. The low temperature part of curve 3 is very similar to the sample denatured by heating (curve 2). From the comparison of curves 2 and 3, it follows that the denaturation caused by freezing is not complete in this solution ($\theta_{den} \approx 0.6$). It is approximately the same as for the sample in the pH 7 solution that is heated to 95° C and quickly cooled ($\theta_{den} \approx 0.7$).

The degree of denaturation is only slightly dependent on the temperature of freezing if the time of incubation in the frozen state is longer than 30 minutes. Melting curve 3 in Figure 4 does not change if the freeze-thaw procedure is followed by incubation at 25° C for 24 hours.

Quick-freezing in liquid nitrogen gave almost the same results as slow freezing at -2° C. Melting a DNA sample at pH 7 after freezing and thawing it in an alkaline medium also revealed denaturation (results not shown).

The influence of Na_2CO_3 on intact DNA and on the denaturation effect of freezing at pH 10.8 is exhibited in Figure 5. The degree of denaturation is much higher without Na_2CO_3 ($\theta_{den} = 0.86$) than when its concentration is 1 mM ($\theta_{den} \approx 0.6$). If

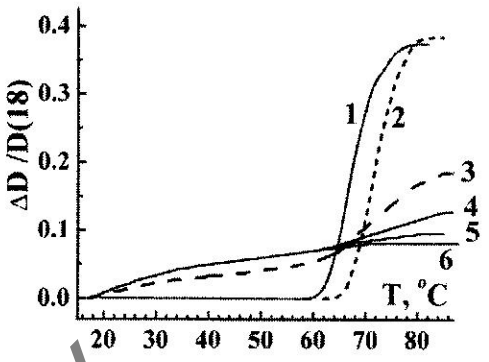

Figure 3: Melting curves of intact DNA (**1**, **2**) and after (**3-6**) heating followed by quick cooling (denatured DNA-2 in Figure 1). The melting curves were registered in alkaline medium (0.1 M NaCl, 0.001 M Na_2CO_3, $5 \cdot 10^{-5}$ M EDTA, pH 10.8.). Heat denaturation was carried out in the same solution at pH 7 or 10.8. **1**, freshly prepared DNA solution; **2**, 24 h incubation at 0 °C or 25 °C. Heating for 10 minutes at **3**, 95 °C, pH 7; **4**, 95 °C, pH 10.8; **5**, 100 °C, pH 7; **6**, 100 °C, pH 10.8.

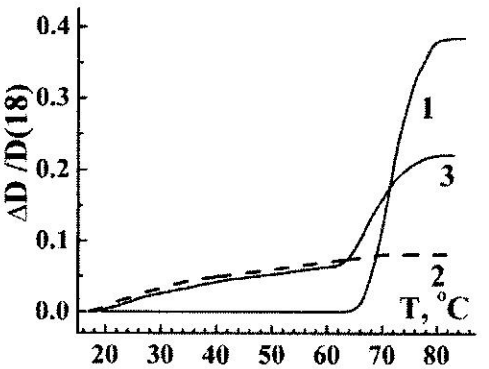

Figure 4: Melting curves for control DNA (**1**), DNA denatured by heating at 100° C and quick cooling (pH 10.8) (**2**), DNA subjected to freezing at -2, -18, -196° C and thawing at 25° C (**3**). DNA heating at 100° C, freezing and melting were carried out in 0.1 M NaCl, 0.001 M Na_2CO_3, $5 \cdot 10^{-5}$ M EDTA, pH 10.8.

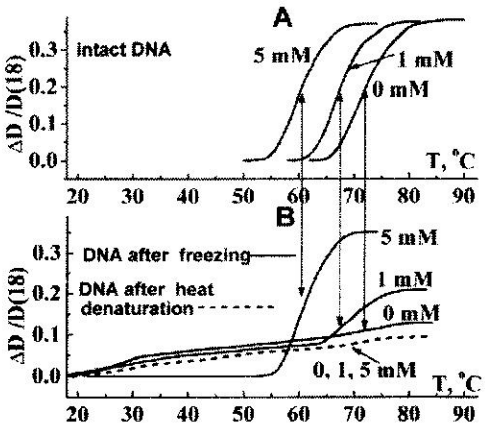

Figure 5: DNA melting curves before (**A**), and after (**B**) freezing (-18° C) and thawing (+25° C) of DNA solution in 0.1 M NaCl, $5 \cdot 10^{-5}$ M EDTA, pH 10.8 at Na_2CO_3 concentrations 0 mM; 1 mM; 5 mM. Coinciding melting curves obtained after heating at 100° C and quick cooling at these Na_2CO_3 concentrations are shown for comparison (**B**).

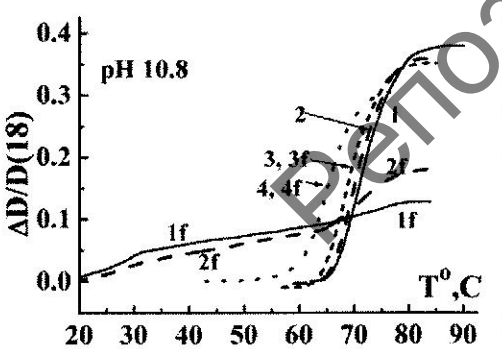

Figure 7: Influence of freezing on platinated DNA. Melting curves were registered in 0.1 M NaCl, $5 \cdot 10^{-5}$ M EDTA, pH 10.8 before (**1-4**) and after (**1f-4f**) freezing. (**1**) No cisplatin ($r = 0$). (**2-4**) 48 h incubation in 0.01M $NaClO_4$ (pH 6.5) at various cisplatin concentrations before freezing (r = cisplatin/P): (**2**) $r = 0.001$; (**3**) $r = 0.01$; (**4**) $r = 0.05$.

Na_2CO_3 concentration increases to 5 mM, then a freeze-thaw cycle in the pH 10.8 solution does not cause any denaturation ($\theta_{den} = 0$) (Figure 5B). Thus, Na_2CO_3 protects DNA against denaturation caused by freezing-thawing. Two observations relating to this protective effect are worth noting: (i) As was shown earlier (25) and as shown in Figure 5A, addition of Na_2CO_3 lowers the melting temperature of the double helix in alkaline medium at fixed pH; and (ii) Na_2CO_3 does not protect DNA against denaturation caused by heating at 100° C (Figure 5B, dashed line). These results imply that Na_2CO_3 influences the degree of denaturation caused by freezing in a way opposite to its influence on heat denaturation.

As follows from Figure 6B, freezing in 5 mM Na_2CO_3 can also give rise to denaturation but at higher pH. There is a small but reproducible decrease in melting temperature after freezing and thawing at pH 11, although θ_{den} is close to zero. At pH 11.25, a prominent denaturation arises ($\theta_{den} \approx 0.85$). Full denaturation occurs in a solution at pH 11.5 (θ_{den} is close to unity). This melting curve (pH 11.5) corresponds to heating a DNA sample at 100° C and quick cooling in ice.

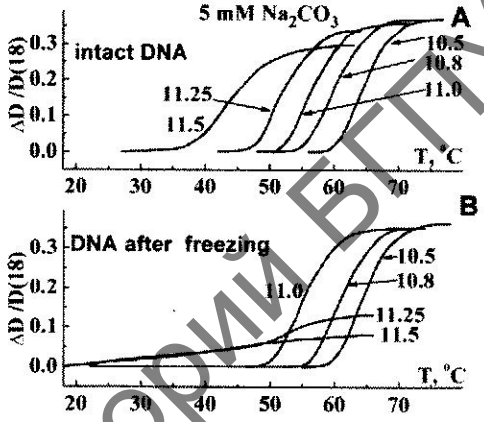

Figure 6: DNA melting curves before (**A**) and after (**B**) freezing. DNA freezing and further melting was carried out in 0.1 M NaCl, 0.005 M Na_2CO_3, $5 \cdot 10^{-5}$ M EDTA at various pH values shown in the figure.

To confirm that denaturation is caused by freezing and thawing in high pH solutions, we have studied the influence of cisplatin on the properties of DNA subjected to freezing in alkaline medium, and compared the impact of this crosslinker with its influence on DNA heated and quickly cooled in ice. It is known that platination gives rise to recovery of the DNA structure after heat denaturation in neutral medium (15). Our previous work (24) indicated that treating DNA with cisplatin decreased its melting temperature to a much greater extent if melting was conducted in alkaline medium. Nevertheless platination gives rise to full recovery of the DNA structure after heat denaturation in alkaline medium (the results are not shown).

The influence of the freezing-thawing procedure on denaturation curves of intact and platinated DNA is shown in Figure 7. To increase the degree of denaturation after freezing-thawing, the procedure was carried out without Na_2CO_3 (in 0.1 M NaCl, $5 \cdot 10^{-5}$ M EDTA, pH 10.8). In this solution, unplatinated DNA is almost fully denatured after freezing and thawing ($\theta_{den} = 0.86$) (Figure 7, curve 1f). At the cisplatin/ nucleotide molar ratio $r = 0.001$, an increase in θ_{max} occurs, although the degree of denaturation is still high (curve 2f, $\theta_{den} = 0.62$). For $r = 0.01$ and 0.05, freezing does not change melting curves [curves 3 and 3f ($r = 0.01$), 4 and 4f ($r = 0.05$)]. Treatment with transplatin gives the same protection against both freezing and heating at 100° C.

Discussion

Structural distortions of biopolymers upon cooling can be caused by a structural failure arisen from water freezing that accompanies cooling (7-9) and by denaturation induced by the decrease in temperature itself and its impact on the enthalpy and entropy of the transition (1-6). A decrease in temperature denatures proteins (1-4) and ribozymes (RNA) (6). Freezing and thawing can give rise to protein denaturation (7),

DNA degradation without denaturation (10-14), and a change in RNA structure (9). In the last case, intramolecular interactions are changed by intermolecular ones.

In this study, we have found that DNA denatures when frozen in alkaline medium. The properties of this denatured DNA are very similar to that which arises under heating and quick cooling (Figure 1). Therefore, a control denaturation was carried out by heating DNA to 95° C or 100° C and quick cooling in ice. Melting curves of DNA previously denatured by heating (Figure 3) were recorded in the alkaline medium used for freezing and thawing experiments (0.1 M NaCl, $5 \cdot 10^{-5}$ M EDTA, 1mM Na_2CO_3, pH 10.8), and in the same solution at pH 7.

As shown in Figure 3 (curve 2), a 24 hour incubation at 0° C or 25° C in the solution used for freezing and thawing does not produce denaturation. However, denaturation takes place when the DNA sample is frozen in this solution (Figure 4, curve 3). The degree of denaturation is almost the same for freezing at -2, -18 and -196° C ($\theta_{den} \approx 0.6$). The melting curves of DNA subjected to freezing and thawing in 0.1 M NaCl, $5 \cdot 10^{-5}$ M EDTA, 1mM Na_2CO_3, pH 10.8 (Figure 4, curve 3) correspond to heating at 95° C for 10 minutes in 0.1M NaCl at pH 7 followed by rapid cooling in ice ($\theta_{den} \approx 0.7$) (Figure 3, curve 3). For freezing in the same solution but without Na_2CO_3 (0.1 M NaCl, $5 \cdot 10^{-5}$ M EDTA, pH 10.8) (Figure 5B, curve 0), the degree of denaturation corresponds to 10 minutes heating at 100° C followed by quick cooling (Figure 5B, dashed line).

It is known that pH of solutions and pK of DNA nitrogen bases change with temperature (26). Denaturation may be caused by a strong increase in pH of solution and/or decrease in pK of nitrogen bases upon sample cooling. However, the melting temperature in media used is higher than 60° C at pH 10.8 (Figure 5A). Therefore, a change in these parameters on cooling must be substantial to cause denaturation. To prove that freezing (and not cooling) gives rise to denaturation, the DNA solution was incubated for 24 hours at 0° C without freezing, and at -2° C (with freezing). No denaturation was found without freezing, and melting curves corresponding to 0 and 25° C coincide (Figure 3, curve 2). However, freezing at almost the same temperature (-2° C) gives rise to denaturation (Figure 4, curve 3). These results demonstrate that freezing is the cause of denaturation. A 2° C reduction in temperature is not likely to produce a large change in pH or cold denaturation.

The freeze induced denaturation strongly depends on the Na_2CO_3 concentration (Figure 5). The freeze-thaw procedure produces much stronger denaturation without Na_2CO_3 than with 1mM Na_2CO_3. The effect of increasing Na_2CO_3 concentration from 1 mM to 5 mM is more prominent: freezing a DNA solution with 5 mM Na_2CO_3 does not cause any denaturation. It must be emphasized that Na_2CO_3 itself destabilizes DNA in alkaline medium [(20) and Figure 5A]. In addition, Na_2CO_3 does not decrease the degree of denaturation caused by heating and quick cooling (Figure 5B). These results mean that the protection provided by Na_2CO_3 against denaturation under freezing is not caused by DNA stabilization.

Denaturation can occur in the presence of 5 mM Na_2CO_3, but only at higher pH values (Figure 6B). At pH 11, freezing gives rise to a small decrease in melting temperature without an increase in θ_{den}, which stays close to zero. A prominent denaturation arises at pH 11.25. At pH 11.5, the degree of denaturation caused by freezing corresponds to 100° C heating and quick cooling (compare with curves 5 and 6 in Figure 3).

Since the strongest denaturing influence of freezing DNA occurred without Na_2CO_3, a 24 hour DNA incubation at 0° C was repeated without it, *i.e.*, in 0.1 M NaCl, $5 \cdot 10^{-5}$ M EDTA, pH 10.8. No cold denaturation was found (data not shown). This confirms that freezing and not cooling causes the denaturation. In contrast to Na_2CO_3, the role of NaCl in the freeze denaturation is not important. A decrease

in NaCl concentration from 0.1 M to 0.01 M only slightly influences the degree of the denaturation (data not shown).

To confirm that a freeze-thaw procedure causes DNA denaturation the same experiments were repeated for DNA chemically modified with cisplatin and transplatin. It is known that both compounds cause DNA renaturation after complete heat denaturation (15) because they form interstrand crosslinks (Figure 2). However, only 6% of bound cisplatin form interstrand crosslinks (16, 17). Other modifications are mainly intrastrand crosslinks which account for ~90% of the modifications (27). They destabilize the double helix. Therefore, cisplatin's overall effect is to decrease a DNA's melting temperature. In addition, the amount of destabilization caused by a fixed amount of platination is larger in alkaline medium used in our study (24). Nevertheless, at $r = 0.001$, the degree of denaturation after freezing slightly decreases (compare curves 1f and 2f in Figure 7). At $r = 0.01$ and $r = 0.05$, the melting curves recorded before and after freezing are almost the same. This means that the distortions caused by freezing disappear after thawing due to interstrand crosslinks formed by cisplatin (Figure 2). This is not a result of general stabilization by cisplatin because it reduces the melting temperature (Figure 7, curves 2-4). This protective influence of platination on frozen DNA implies that freezing in alkaline medium causes DNA denaturation similar to that which arises by heating. The denaturation caused by freezing is reversible in DNA with a sufficient number of interstrand crosslinks. A value of $r = 0.01$ corresponds to one interstrand crosslink per 1500 nucleotides. DNA used in this study is 30 to 50 kb in length, thus there are 40 to 60 interstrand crosslinks per DNA molecule. This quantity is sufficient to restore DNA structure after freezing in alkaline medium. However, 4-6 interstrand crosslinks per DNA molecule at $r = 0.001$ is not sufficient for full renaturation upon thawing although a partial restoration is observed. Incomplete renaturation for $r = 0.001$ can arise from single-strand breaks. Similar results were obtained for transplatin. The protective influence of cisplatin against freezing-thawing (as against heating-cooling) demonstrates that this procedure causes DNA denaturation.

What are the possible causes of DNA denaturation upon freezing it in alkaline medium? First, it is known that the composition of a solution and its pH can be changed by freezing (8, 28). However, we have demonstrated that freezing does not change pH at a "macrolevel" in the solutions used for our studies, and the pH value is conserved after freezing 70-80% of the solution and moving the ice away (data not shown). Second, the structure of ice in alkaline medium strongly differs from the structure at neutral pH (29-31). This may influence DNA in alkaline medium. However, the structure of ice changes at a temperature much lower than that was used in our study (29-31). Besides, this assumption does not explain a decrease of denaturation at higher Na_2CO_3 concentrations for the solution at a given pH. The third, and most likely mechanism is based on observations that suggest strong changes in pH can occur at the "microlevel" during the process of freezing. At subzero temperatures, pockets of unfrozen water can form within the ice that contain the macromolecules and ions that do not fit into the ice lattice (32). The increase in pH in these pockets due to concentration changes may cause the denaturation observed in this work (Figure 8). Na_2CO_3 is the main buffering component of solutions used for freezing-thawing experiments. Na_2CO_3 neither decreases the degree of denaturation caused by heating and quick cooling (Figure 5B, dashed curve) nor increases the melting temperature (Figure 5A). However, Na_2CO_3 increases the buffer capacity of the solution. This higher buffer capacity can be expected to reduce the elevation of pH value in the liquid pockets during freezing and make this elevation insufficient for DNA denaturation under freezing.

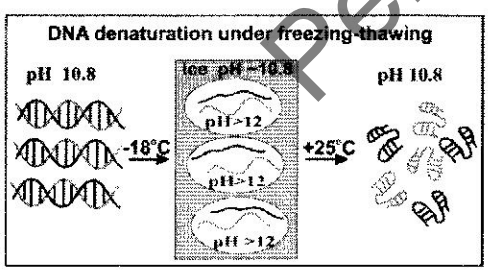

Figure 8: DNA denaturation caused by freeze-thaw procedure.

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References and Footnotes

1. G. Graziano, F. Catanzano, A. Riccio, and G. Barone. *J Biochem 122*, 395-401 (1997).
2. G. Caldarelli and P. De Los Rios. *Journal of Biological Physics 27*, 229-241 (2001).
3. A. Hansen, M. Jensen, K. Sneppen, and G. Zocchi. *Eur Phys J B 6*, 157-161 (1998).
4. A. Hansen, M. Jensen, K. Sneppen, and G. Zocchi. *Eur Phys J B 10*, 193-196 (1999).
5. R. H. Hatley and F. Franks. *FEBS Lett 257*, 171-173 (1989).
6. P. J. Mikulecky and A. L. Feig. *J Am Chem Soc 124*, 890-891 (2002).
7. E. Cao, Y. Chen, Zh. Cui, P. R. Foster. *Biotechnology and Bioengineering 82*, 684-690 (2003).
8. G. Astl and E. Mayer. *Biochim Biophys Acta 1080*, 155-159 (1991).
9. X. Sun, J. M. Li, and R. M. Wartell. *RNA 13*, 2277-2286 (2007).
10. V. N. Lyscov, E. A. Konopankina, L. F. Malisheva, and Y. Moshkovsky. *Biophysics (Mosc) 11*, 726-728 (1966).
11. V. N. Lyscov and Y. Moshkovsky. *Biochim Biophys Acta 190*, 101-110 (1969).
12. V. N. Lyscov. *Biochim Biophys Acta 190*, 111-115 (1969).
13. W. H. J. Crombach. *Antonie van Leeuwenhoek 39*, 249-255 (1973).
14. J.-H. Park, Ch.-K. Hyun, S.-K. Jeong, M.-A. Yi, S.-T. Ji, and H.-K. Shin. *International Journal of Food Science & Technology 35*, 555-561 (2000).
15. J. L. Butour and J. P. Macquet. *Biochim Biophys Acta 653*, 305-315 (1981).
16. V. Brabec and M. Leng. *Proc Natl Acad Sci 90*, 5345-5349 (1993).
17. O. Vrana, V. Boudny, and V. Brabec. *Nucleic Acids Res 24*, 3918-3925 (1996).
18. D. Y. Lando, V. P. Egorova, V. I. Krot, and A. A. Akhrem. *Molecular Biology 30*, 701-706 (1996) [Russia, *Engl Transl 29*, 418-421 (1996)].
19. R. Zaludova., V. Kleinwachter, and V. Brabec. *Biophysical Chemistry 60*, 135-142 (1996).
20. V. Kleinwachter and H. Rau. *Studia Biophysica 103*, 5-12 (1984).
21. O. Vrana, V. Brabec, and V. Kleinwachter. *Anticancer Drug Design 1*, 95-109 (1986).
22. O. Novakova, O. Vrana, V. I. Kiseleva, and V. Brabec. *Eur J Biochem 228*, 616-624 (1995).
23. K. Stehlikova, J. Kasparkova, O. Novakova, A. Martinez, V. Moreno, and V. Brabec. *FEBS J 273*, 301-314 (2006).
24. E. N. Galyuk, A. S. Fridman, V. I. Vorob'ev, S. G. Haroutiunian, S. A. Sargsyan, M. M. Hauruk, and D. Y. Lando. *J Biomol Struct Dyn 25*, 407-418 (2008).
25. E. N. Galyuk, D. Y. Lando, V. P. Egorova, H. Dai, and Yu. M. Dosin. *J Biomol Struct & Dynam 20*, 801-809 (2003).
26. N. Kochetkov *et al. Organic Chemistry of Nucleic Acids*, Plenum Press, New York (1972).
27. A. Eastman. *Pharmacol Ther 34*, 155-166 (1987).
28. G. Gómez, M. J. Pikal, and N. Rodríguez-Hornedo. *Pharm Res 18*, 90-97 (2001).
29. E. A. Zheligovskaya and G. G. Malenkov. *Russian Chemical Reviews 75*, 64-85 (2006).
30. T. Matsuo, Y. Tajima, and H. Suga. *J Phys Chem Solids 47*, 165-173 (1986).
31. A. J. Leadbetter, R. C. Ward, J. W. Clark, P. A. Tucker, T. Matsuo, and S. Suga. *J Chem Phys 82*, 424-428 (1985).
32. Franks, F. *Biophysics and biochemistry at low temperatures*. Cambridge University Press, Cambridge, UK (1985).

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