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Rapid renaturation of complementary DNA strands mediated by cationic detergents: A role for high-probability binding domains in enhancing the kinetics of molecular assembly processes

(annealing/nucleic acid hybridization/dodecyltrimethylammonium bromide/cetyltrimethylammonium bromide)

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ABSTRACT **The rate of renaturation for complementary DNA strands can be enhanced $>10^4$-fold by the addition of simple cationic detergents, and the reaction is qualitatively and quantitatively very similar to that found with purified heterogeneous nuclear ribonucleoprotein A1 protein. Under optimal conditions, renaturation rates are >2000-fold faster than reactions run in 1 M NaCl at 68°C. The reaction is second-order with respect to DNA concentration, and reaction rates approach or equal the rate with which complementary strands are expected to encounter each other in solution. Renaturation can even be observed well above the expected melting temperature of the duplex DNA, demonstrating that some cationic detergents have DNA double-helix-stabilizing properties. The reaction is also extremely rapid in the presence of up to a 10^6-fold excess of noncomplementary sequences, establishing that renaturation is specific and relatively independent of heterologous DNA. This finding also implies that up to several thousand potential target sequences can be sampled per strand per second. Such reagents may be useful for procedures that require rapid nucleic acid renaturation, and these results suggest ways to identify and design other compounds that increase the kinetics of association reactions. Moreover, this work provides further support for a model relating the existence of flexible, weakly interacting, repeating domains to their function in rapid molecular assembly processes *in vitro* and *in vivo*.**

The renaturation of nucleic acid strands is of fundamental importance in biological processes such as genetic recombination and repair (1, 2). In addition, the ability to renature complementary DNA strands *in vitro* (3–5) has become a widely used tool for the identification and analysis of specific nucleic acid sequences (6–15). For these and other reasons, considerable effort has gone towards understanding the mechanism of nucleic acid renaturation, as well as towards finding conditions where the rate of renaturation is enhanced (16–21).

Recently, we and others demonstrated that the A1 protein of heterogeneous nuclear ribonucleoprotein (hnRNP) can rapidly renature complementary strands of both DNA and RNA (refs. 22 and 23; X. Dong and S. Munroe, personal communication). This unanticipated property of A1 was accounted for by its ability to bind to nucleic acid strands and present flexible, weakly interacting domains with a repeating structure (22). This model predicts that other molecules possessing flexible domains containing, for example, repeating hydrophobic residues, will also substantially increase the rate of nucleic acid renaturation when they are bound to complementary sequences. To test this hypothesis, as well as to identify readily available compounds that could be used to accelerate nucleic acid renaturation, we investigated the properties of two simple cationic detergents, dodecyl- and cetyltrimethylammonium bromide (DTAB and CTAB).

DTAB and CTAB are variants of the quaternary amine tetramethylammonium bromide (TMAB) in which one of the methyl groups is replaced by either a 12-carbon (DTAB) or a 16-carbon (CTAB) alkyl group. TMAB is the bromide salt of the tetramethylammonium ion, a reagent used in nucleic acid renaturation experiments to decrease the G·C-content bias of the melting temperature (24, 25). DTAB and CTAB are similar in structure to sodium dodecyl sulfate (SDS), with the replacement of the negatively charged sulfate of SDS by a positively charged quaternary amine. While SDS is commonly used in hybridization buffers to reduce nonspecific binding and inhibit nucleases, it does not greatly affect the rate of renaturation.

This paper shows that DNA renaturation can be enhanced $>10^4$-fold by the cationic detergents DTAB and CTAB. The renaturation reactions are second-order with respect to DNA concentration, and the rate constants are consistent with a mechanism that is limited by the rate at which the complementary strands encounter each other in solution. For CTAB, the reaction rate is maximal at around 75°C, although renaturation can be observed even at temperatures well above the melting temperature expected for the double-stranded DNA in the absence of detergent. Renaturation is relatively insensitive to detergent and heterologous DNA concentrations over several orders of magnitude. These results indicate that cationic detergents may be useful as reagents to promote rapid nucleic acid renaturation. In addition, our results support a mechanism, initially postulated for A1 hnRNP protein (22), that may be relevant to the general problem of how rapid and specific molecular assembly can be achieved.

MATERIALS AND METHODS

DNA Substrates. A 124-nucleotide ^{32}P-labeled DNA fragment, which served as the substrate for the renaturation reactions, was prepared as described (22). Other DNA substrates (for Fig. 5) were similarly prepared from various restriction fragments of plasmid pSV2gpt (26). DNA concentrations are expressed in nucleotides.

Reagents. DTAB, CTAB, TMAB, and salmon sperm DNA were purchased from Sigma.

Renaturation Reaction Conditions. Unless otherwise noted, detergent-mediated reactions were carried out in 10 μl of 10 mM Tris·HCl (pH 7.5) containing 1 mM EDTA, 1 nM ^{32}P-labeled DNA substrate, and NaCl as indicated. With the

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Abbreviations: DTAB, dodecyltrimethylammonium bromide; CTAB, cetyltrimethylammonium bromide; TMAB, tetramethylammonium bromide; hnRNP, heterogeneous nuclear ribonucleoprotein.

exception of the NaCl and cationic detergent, all components were mixed, incubated at 95°C for 5 min, and then rapidly chilled in ice water to generate single strands. NaCl was added, and the mixtures were preincubated at the appropriate temperature for 2 min before the addition of detergent. Reactions were stopped with SDS by the addition of 30 μl of 10 mM Tris, pH 7.5/1 mM EDTA/0.1% SDS/3% glycerol/0.03% bromophenol blue. For Figs. 1–3, the stop buffer also included 100 mM NaCl. After termination, the reaction mixtures were incubated for 1 min at 75°C, cooled to room temperature, extracted with phenol/chloroform, 1:1 (wt/vol), to remove the detergent, and electrophoresed in polyacrylamide gels under nondenaturing conditions to separate the single-stranded and double-stranded forms of the substrate DNA. Comparable results were obtained when reactions were stopped without including the phenol/chloroform extraction (data not shown). Gels were then vacuum-dried and subjected to autoradiography, and the amount of DNA in each band was quantitated by densitometry. Reactions carried out in the presence of monovalent cation alone were performed as described (22), using 100 nM DNA substrate.

RESULTS

The detergent-mediated renaturation reactions proceed to completion following second-order kinetics. Thus, a plot of the natural logarithms of the initial half-times of renaturation against the natural logarithms of the initial DNA concentrations (in nucleotides) in the presence of either 1 mM DTAB or 1 mM CTAB yields a line whose slope is −1.0 (Fig. 1). The second-order rate constants for renaturation of the DNA substrate were determined in the presence of DTAB, CTAB, 1 M TMAB, and in the absence of detergent. The rate constant for CTAB at 68°C is 2.5×10^7 $M^{-1}\cdot sec^{-1}$, which is about 5 times greater than the rate constant for DTAB. This rate is $>10^4$-fold faster than reactions run under similar conditions in the absence of detergent and about 700-fold faster than reactions run in 1 M TMAB (Fig. 1) or 1 M NaCl (22). Interestingly, the second-order rate constant for A1 hnRNP protein-mediated renaturation reactions at 60°C is 10^7 $M^{-1}\cdot sec^{-1}$ (22), demonstrating that the renaturation properties for DTAB, CTAB, and A1 are similar both qualitatively and quantitatively.

The dependence of the renaturation kinetics on the concentration of DTAB, CTAB, and TMAB is shown in Fig. 2 (Because the rate constants and concentrations being compared differ greatly, data in Figs. 2–4 are plotted on logarithmic or semilogarithmic scales.) In reactions performed in the absence of detergent, the second-order renaturation rate constant is around 700 $M^{-1}\cdot sec^{-1}$ (dashed line). The concentrations at which DTAB and CTAB promote renaturation are

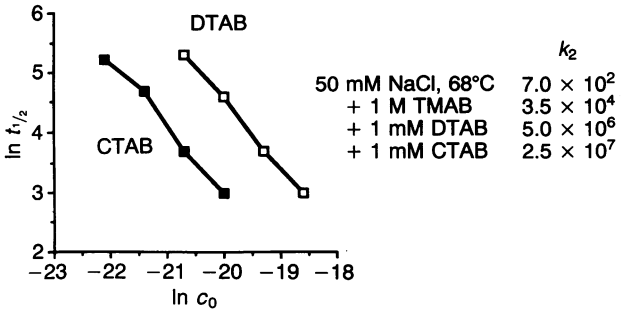

FIG. 1. Kinetics of uncatalyzed, 1 mM DTAB (□)-, 1 mM CTAB (■)-, and 1 M TMAB-mediated renaturation. Reactions were performed at 68°C in 50 mM NaCl as described in *Materials and Methods*. Second-order association rate constants in $M^{-1}\cdot sec^{-1}$ were calculated by using the equation $k_2 = c_0^{-1}\cdot t_{1/2}^{-1}$.

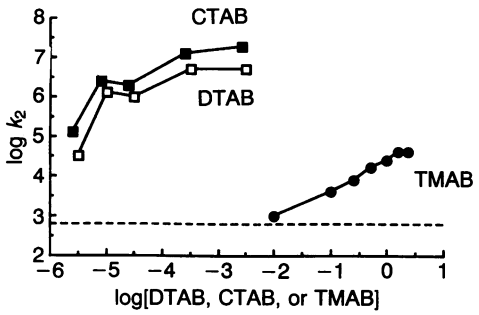

FIG. 2. Rates of DTAB (□)-, CTAB (■)-, and TMAB (●)-mediated renaturation as a function of the detergent or cation concentration. Reactions were performed as described in Fig. 1. The dashed line indicates the kinetics of the uncatalyzed reaction.

in the micromolar range, and their activities are near maximal at about 0.1–1 mM. CTAB is about 5 times more effective than DTAB at all concentrations tested. In contrast, TMAB does not begin to promote renaturation until its concentration reaches 10–100 mM (NaCl was included in all reaction mixtures at 50 mM), and a maximum is reached at about 1 M. The behavior of TMAB in this respect is similar to that of other monovalent cations and demonstrates the importance of the carboxyl tail of DTAB and CTAB in promoting renaturation. These results indicate that relatively small quantities of detergent are sufficient to facilitate renaturation and that excess detergent in solution does not profoundly alter the renaturation kinetics. These results resemble those found with A1 hnRNP protein (22) and are in striking contrast to the much higher concentration of phenol (20), simple monovalent cation (16), or standard volume-excluding agent such as PEG (19) or dextran sulfate (27) required to promote renaturation. Because both detergents have renaturation activity at concentrations well below their critical micelle concentrations and because they promote renaturation at similar concentrations even though the critical micelle concentration for CTAB is lower than it is for DTAB (28), these results also indicate that the presence of micelles in solution is not required for renaturation.

The rate of renaturation for CTAB and TMAB as a function of temperature is shown in Fig. 3. The presence of 1 mM CTAB enhances renaturation over a broad range of temperatures and roughly parallels the effects of 1 M TMAB, with the exception that the CTAB-mediated reactions are about 500-fold faster. This is surprising, as the melting temperature for the DNA substrate under the same conditions in the absence of CTAB is 78°C (data not shown). This indicates that even 1 mM CTAB is strongly helix-stabilizing and suggests that CTAB may shield DNA phosphate charges or bind more tightly to duplex than to single-stranded DNA. These results indicate that a helix-destabilizing property is not required for the renaturation activity of CTAB, in con-

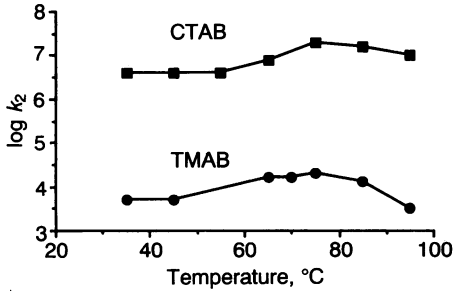

FIG. 3. CTAB-mediated renaturation as a function of temperature. Reactions with 1 mM CTAB (■) and 1 M TMAB (●) were performed as in Fig. 1.

trast to what has been proposed for the *Escherichia coli* SSB and phage T4 gene *32* proteins, where helix-destabilizing properties are believed to help mediate renaturation by melting-out intrastrand secondary structures (29, 30).

We have examined the effects of increasing amounts of noncomplementary, single-stranded salmon sperm DNA on the ability of CTAB to renature the 124-nucleotide-long DNA strands (Fig. 4). These measurements were made in the presence of 0.1, 1, or 10 mM CTAB and compared with reactions run at 68°C in 1 M NaCl. As expected (31), salmon sperm DNA at concentrations of up to 1 mM in nucleotide (300 μg/ml) has little effect on the kinetics of renaturation of the 124-nucleotide substrate at 68°C in 1 M NaCl. In contrast, added salmon sperm DNA affects the kinetics of renaturation in the presence of CTAB, although the maximal rates of renaturation for all CTAB concentrations tested are indistinguishable. With 0.1 mM CTAB, renaturation is stimulated 6-fold by the addition of 0.01 mM salmon sperm DNA and then inhibited as additional DNA is added. With 1 mM CTAB, a similar result is obtained, with inhibition occurring only at higher salmon sperm DNA concentrations. When reactions are run in 10 mM CTAB, reactions are somewhat less effective in the absence of heterologous DNA. As more salmon sperm DNA is added, renaturation is stimulated, and then inhibited, and this inhibition occurs to the same extent and at the same concentration of salmon sperm DNA as it does for 1 mM CTAB. Salmon sperm DNA present at 1 mM represents a 10^6-fold excess of nonhomologous DNA with respect to the substrate strands, which are present at 1 nM. A k_2 of 8×10^7 M^{-1}·sec^{-1} for renaturation in the presence of 1 mM CTAB, 1 nM labeled substrate DNA, and 0.1 mM salmon sperm DNA represents a half-time for renaturation of 12 sec. These results demonstrate that renaturation between strands is sequence-specific and that a large number of potential target sequences can be sampled in a short period of time.

Fig. 5 shows that the relative rate of CTAB-mediated renaturation, when compared with standard conditions (68°C, 1 M NaCl), varies with the DNA strand length. Under these conditions, CTAB is most effective in promoting the renaturation of short strands. As the lengths of the DNA strands increase, the relative enhancement is reduced from around 300-fold (124-nucleotide strands) to around 20-fold (1800-nucleotide strands), although the extent of these differences is a function of the temperature and NaCl concentration (unpublished results). At 68°C in 1 M NaCl, the second-order rate constant (when measured in moles of nucleotide) decreases with the square root of the strand length (when strands do not contain repeated sequences) (16). These results indicate that the rate of CTAB-mediated renaturation is more severely affected by increasing strand length, perhaps due to excluded-volume effects (16) or some other

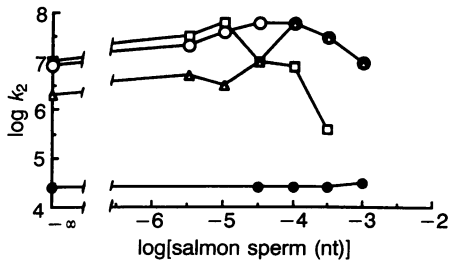

FIG. 4. Effect of heterologous, single-stranded DNA on the detergent-mediated renaturation kinetics. Renaturation was carried out in 0.1 mM (□), 1 mM (○), and 10 mM (△) CTAB and in 1 M NaCl (●) in the presence of various concentrations of salmon sperm DNA that had been sonicated to an average length of 300 nucleotides (nt). Reactions with CTAB were at 75°C in 400 mM NaCl.

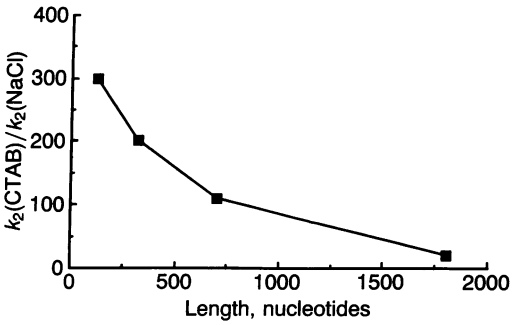

FIG. 5. Relative enhancement of renaturation in 1 mM CTAB with respect to reactions carried out in 1 M NaCl at 68°C, as a function of DNA strand length. Reactions with CTAB were at 75°C in 400 mM NaCl.

property of longer strands (see *Discussion*). Similar results were obtained for A1 protein. Moreover, for both A1-mediated (22) and CTAB-mediated (unpublished results) renaturation between short and long strands, the observed kinetics are closer to those expected for the shorter complementary partner.

DISCUSSION

In an earlier report, we proposed a model to account for the unexpected ability of A1 hnRNP protein to promote nucleic acid renaturation (22). We suggested that A1 mediates renaturation primarily by (*i*) binding to DNA and (*ii*) presenting flexible, repeating domains, composed of hydrophobic residues and charged groups, which interact with high probability with similarly coated DNA strands in solution. In this model, these domains provide weak binding energy whenever two appropriately coated nucleic acids collide in solution. Such domains would be able to interact in a wide variety of conformations, thereby increasing the probability of binding, as well as the ability of the initial complex to undergo rapid conformational changes prior to dissociation. Such conformational changes, mediated by thermal fluctuation, would allow the strands to sample a large selection of possible interactions between strands prior to dissociation, increasing the likelihood of a nucleation event. In addition, binding energy could be provided at a considerable distance when compared with the association of complementary base pairs, further increasing the probability of an interaction. In this way, associations via high-probability binding domains are thought to lower the activation energy for nucleating the two strands, thereby increasing the kinetics of renaturation.

To explore the minimal predictions for this model (i.e., the necessity for nucleic acid-binding capability and the existence of domains able to interact weakly in a variety of conformations), we tested selected cationic detergents for their ability to promote nucleic acid renaturation. Whereas one A1 hnRNP protein provides a single, 120-amino acid, flexible repeating unit every 12 nucleotides (32), CTAB is postulated to bind along the DNA molecule with one CTAB molecule bound per DNA phosphate. Unlike polypeptides, cationic detergents cannot form amphipathic α-helices or other specific secondary structures, ruling out the possibility that such motifs are required for renaturation activity.

Our present results demonstrate that the simple cationic detergents DTAB and CTAB can enhance the kinetics of renaturation of complementary DNA strands $>10^4$-fold when compared with reactions run in the absence of detergent and that, under optimized conditions, CTAB-mediated renaturation is >2000-fold faster than reactions in 1 M NaCl at 68°C. The renaturation kinetics in the presence of the two detergents are qualitatively and quantitatively similar to that found

with the A1 hnRNP protein (22). Renaturation is sequence-specific, since the kinetics and extent of the reaction are not inhibited by the presence of up to a 10^6-fold excess of noncomplementary sequences. Moreover, CTAB-mediated renaturation can occur well above the melting temperature of the duplex DNA, indicating that this detergent has DNA helix-stabilizing properties. These findings are consistent with the view that cationic detergents enhance the kinetics of renaturation by a mechanism similar to that proposed for A1 hnRNP protein (22).

Mechanistic Implications. In general, the maximal rate at which two molecules can associate in solution is limited by the rate at which they encounter each other as they diffuse. This limit is often referred to as the "diffusion limit," although reactions can be considerably slower while still being limited by the rate of diffusion (33, 34). Estimated values for this limit range from 10^8 to 10^9 $M^{-1}\cdot sec^{-1}$ for macromolecular association at 25°C (34). Values for the CTAB-mediated renaturation of nucleic acid strands in the presence of salmon sperm DNA at 75°C are about 8×10^7 $M^{-1}\cdot sec^{-1}$, but these values are measured in moles of nucleotide. Once converted into moles of strand, the macromolecular association rate constant is around 10^{10} $M^{-1}\cdot sec^{-1}$. This value is consistent with, or in excess of, the theoretically predicted encounter limit, even when one considers the increased rate of diffusion expected at these elevated temperatures. This suggests that the initial short-lived associations between strands, which are mediated by CTAB, are sufficient to make a productive nucleation event likely before dissociation occurs.

Interestingly, the effect of strand length on the rate of renaturation (Fig. 5) is much less apparent when the macromolecular association rate is considered. For 1800-nucleotide-long strands, this rate is around 2.5×10^9 $M^{-1}\cdot sec^{-1}$, only 4-fold less than that found for 124-nucleotide strands. This indicates that the renaturation of longer strands may also be encounter-limited. To test this hypothesis, the diffusion constants of the substrate nucleic acids must be measured under various conditions to determine whether the values obtained correlate with the kinetics of renaturation.

One can estimate the time which non-base-paired strands remain associated during an encounter, by using data from Fig. 4. Because the nonhomologous DNA strands are about twice as long as the probe strands and one expects a probe strand to encounter about half of the nonhomologous strands before a homologous one is encountered, an encounter rate of about 25,000 strands every 12 sec (the observed half time for renaturation under these conditions) in the presence of 1 mM CTAB and 0.1 mM salmon sperm DNA can be derived. This estimate gives a rate of >2000 strands encountered per substrate strand per second (or 0.5 msec per interaction), indicating that interactions between strands that have not yet renatured are extremely short-lived. If the reactions are encounter-limited, this value also sets an upper limit on the maximum interaction time required to ensure a productive nucleation event when complementary sequences are present on the interacting strands.

Nonhomologous DNA can either increase or decrease the rate of renaturation in the presence of CTAB, depending on the conditions. When CTAB is limiting with respect to the nucleotide concentration, inhibition could simply be due to a requirement for a CTAB-coated nucleic acid strand. When sufficient CTAB is present, even a 10^5-fold excess (in nucleotides) of noncomplementary genomic DNA (with respect to the substrate strands) does not inhibit the kinetics of renaturation, although additional DNA is inhibitory. The inhibition observed at high levels of nonhomologous DNA suggests that the renaturation rate may eventually be limited by the rate of dissociation from noncomplementary strands. Alternatively, sufficient noncomplementary DNA may disrupt an association between complementary strands before nucleation occurs. Indeed, binding domains with multiple potential binding conformations would be particularly prone to such inhibition due to facilitated exchange between strands.

The weak dependence for CTAB-mediated renaturation on the concentration of detergent (between 10 μM and 10 mM) or heterologous DNA (over a 10^6-fold range) is significantly different from the histone or first-order RecA-mediated renaturation reactions (35–39), which are thought to proceed via the formation of DNA/protein aggregates. Although CTAB is known to cause the aggregation and precipitation of DNA at temperatures near 0°C (40), renaturation is inhibited under such conditions, particularly in the presence of nonhomologous DNA (unpublished results). This indicates that CTAB causes aggregation at temperatures different from those at which it promotes renaturation, although both of these properties may be due to the same attributes (i.e., the ability to bind to DNA through charge–charge interactions and associate through hydrophobic forces). Unlike RecA-mediated renaturation, the CTAB-mediated reaction is second-order with respect to the concentration of complementary strands in solution. In addition, aggregation-mediated renaturation would not be expected to take place at the encounter limit, which is based on the rate of diffusion in solution. For aggregation-mediated reactions, renaturation should decrease as the ratio of complementary strands to noncomplementary strands decreases, provided that association within the aggregate is the rate-limiting step. These differences suggest that the effects of CTAB and heterologous DNA (below 0.1 mM) are due to causes other than aggregation, such as, perhaps, excluded-volume effects, changes in the rate of diffusion of the complementary strands, or changes in the concentration of free CTAB.

Relationship to Other Molecular Assembly Processes. This, and previous (22), work directly demonstrates that the rate of association for a specific binding interaction (base pairing) can be increased several orders of magnitude by the presence of a separate, relatively nonspecific, repeating domain. Because these domains are simple and because the mechanism of this reaction is thought to overcome a general barrier to the formation of specific macromolecular complexes, one might expect that structurally similar domains would have evolved readily during evolution to facilitate other biologically important association reactions. Indeed, in addition to nucleic acids themselves, many macromolecules involved in processes such as transcription (41–45), protein trafficking (46–49), and RNA processing (50, 51) contain apparently flexible domains composed of different repeating chemical constituents, although the functions of these domains are still only poorly understood. Our work is consistent with the view that these domains function by increasing the rate at which specific association reactions occur and provides a mechanism to account for this effect.

There are also other reactions in which an initially nonspecific binding interaction, mediated by a repeating motif, is thought to increase the association kinetics of a specific complex via the stabilization of a binding intermediate. A well-studied example of this is the process by which some DNA-binding proteins locate their specific binding sites (52–54). This process relies on nonspecific electrostatic interactions to facilitate one-dimensional diffusion and intrasegment strand transfer. In addition, evidence is accumulating that nonspecific hydrophobic interactions are important contributors to the rapid kinetics of protein folding (55–58). Such similarities in association reactions that are otherwise unrelated may indicate that initial, nonspecific interactions mediated by either hydrophobic residues or charged groups may be a general feature of rapid association reactions. Because CTAB increases the thermostability of the DNA duplex, it may also help stabilize the final, double-stranded DNA complex. For protein folding, hydrophobic groups are known to stabilize protein structure by making specific interactions in the hydrophobic core. In contrast, the equilibrium of DNA/DNA-binding

protein interactions is not affected by increases in DNA strand length, so long as the rates of both association and dissociation of the complex are enhanced (59). Such interactions, which increase the kinetics of both association and dissociation, would be particularly useful for the rapid turnover of intermediate complexes in biological processes such as transcription, translation, and splicing.

While CTAB can increase the rate of nucleic acid renaturation *in vitro*, simple hydrophobic domains might not lead to enhanced association *in vivo*, because macromolecules are already highly concentrated under these conditions (60). More specific interactions might then be required, and could be achieved if different types of repeating domains were used for components involved in different biological processes. Appropriately matched high-probability binding domains might even allow for factors involved in a particular biological process to concentrate into specific subdomains of the cell, where they could rapidly locate new substrates after dissociating from processed ones. Indeed, the high concentrations of particular splicing components that are observed in different regions of the nucleus (61–64) may stem from high-probability, short-lived interactions among macromolecules, such as A1 hnRNP protein, that possess reactive domains of the type we have suggested.

Practical Implications. These results indicate that cationic detergents, in addition to A1 hnRNP protein, could be extremely useful in experiments where increasing the rate of nucleic acid renaturation is desired. If the proposed mechanism for renaturation is correct, one should also be able to design and identify domains that increase the association kinetics of many other reactions that are not presently limited by their rate of encounter or a subsequent chemical step. Through the attachment of high-probability binding domains to macromolecules such as nucleic acids, enzymes, ribozymes, and antibodies, it may be possible to increase the association kinetics of a variety of reactions of biological, medical, and industrial importance.

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1. Stahl, F. W. (1979) *Genetic Recombination* (Freeman, San Francisco).
2. Freidberg, E. C. (1985) *DNA Repair* (Freeman, New York).
3. Marmur, J. & Lane, D. (1960) *Proc. Natl. Acad. Sci. USA* **46**, 453–461.
4. Doty, P., Marmur, J., Eigner, J. & Schildkraut, C. L. (1960) *Proc. Natl. Acad. Sci. USA* **46**, 461–476.
5. Marmur, J. & Doty, P. (1961) *J. Mol. Biol.* **3**, 585–594.
6. Schildkraut, C. L., Marmur, J. & Doty, P. (1961) *J. Mol. Biol.* **3**, 595–617.
7. Nygaard, A. P. & Hall, B. D. (1964) *J. Mol. Biol.* **9**, 125–142.
8. Gillespie, D. & Speigelman, S. (1965) *J. Mol. Biol.* **12**, 829–842.
9. Britten, R. J. & Kohne, D. E. (1968) *Science* **161**, 529–540.
10. Gall, J. G. & Pardue, M. L. (1971) *Methods Enzymol.* **21**, 470–480.
11. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
12. Grunstein, M. & Hogness, D. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3961–3965.
13. Berk, A. J. & Sharp, P. A. (1977) *Cell* **12**, 721–732.
14. Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G. & Erlich, H. (1986) *Cold Spring Harbor Symp. Quant. Biol.* **51**, 263–273.
15. Hames, B. D. & Haber, S. J. (1985) *Nucleic Acid Hybridization: A Practical Approach* (IRL, Oxford).
16. Wetmur, J. G. & Davidson, N. (1968) *J. Mol. Biol.* **31**, 349–370.
17. Studier, F. W. (1969) *J. Mol. Biol.* **41**, 189–197.
18. Studier, F. W. (1969) *J. Mol. Biol.* **41**, 198–209.
19. Wetmur, J. G. (1971) *Biopolymers* **10**, 601–613.
20. Kohne, D. E., Levison, S. A. & Byers, M. J. (1977) *Biochemistry* **16**, 5329–5341.
21. Wetmur, J. G. (1976) *Annu. Rev. Biophys. Bioeng.* **29**, 336–361.
22. Pontius, B. W. & Berg, P. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 8403–8407.
23. Kumar, A. & Wilson, S. H. (1990) *Biochemistry* **29**, 10717–10722.
24. Melchoir, W. B. & von Hippel, P. H. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 298–302.
25. Wood, W. I., Gitschier, J., Lasky, L. A. & Lawn, R. M. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1585–1588.
26. Mulligan, R. C. & Berg, P. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2072–2076.
27. Wetmur, J. G. (1975) *Biopolymers* **14**, 2517–2524.
28. Mukerjee, P. & Mysels, K. J. (1971) *Critical Micelle Concentrations in Aqueous Surfactant Systems* (Natl. Bureau Standards, Washington).
29. Christiansen, C. & Baldwin, R. L. (1977) *J. Mol. Biol.* **115**, 441–454.
30. Alberts, B. M. & Frey, L. (1970) *Nature (London)* **227**, 1313–1318.
31. Subirana, J. A. & Doty, P. (1966) *Biopolymers* **4**, 171–187.
32. Cobianchi, F., SenGupta, D. N., Smudzka, B. Z. & Wilson, S. H. (1986) *J. Biol. Chem.* **261**, 3536–3543.
33. Schmitz, K. S. & Schurr, J. M. (1972) *J. Phys. Chem.* **76**, 534–545.
34. Berg, O. G. & von Hippel, P. H. (1985) *Annu. Rev. Biophys. Biophys. Chem.* **14**, 131–160.
35. Cox, M. M. & Lehman, I. R. (1981) *Nucleic Acids Res.* **9**, 389–400.
36. Weinstock, G. M., McEntee, K. & Lehman, I. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 126–130.
37. Keener, S. L. & McEntee, K. (1984) *Nucleic Acids Res.* **12**, 6127–6139.
38. Bryant, F. R. & Lehman, I. R. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 297–301.
39. Bryant, F. R., Menge, K. L. & Nguyen, T. T. (1989) *Biochemistry* **28**, 1062–1069.
40. Geck, P. & Nasz, I. (1983) *Anal. Biochem.* **135**, 264–268.
41. Sigler, P. B. (1988) *Nature (London)* **333**, 210–212.
42. Ptashne, M. (1988) *Nature (London)* **335**, 683–689.
43. Cress, W. D. & Treizenberg, S. J. (1991) *Science* **251**, 87–90.
44. Mitchell, P. J. & Tjian, R. (1989) *Science* **245**, 371–378.
45. Cordon, J. L. (1990) *Trends Biochem. Sci.* **15**, 383–387.
46. von Heijne, G. (1985) *J. Mol. Biol.* **184**, 99–105.
47. Kaiser, C. A., Preuss, D., Grisafi, P. & Botstein, D. (1987) *Science* **235**, 312–317.
48. Bernstein, H. D., Rapoport, T. A. & Walter, P. (1989) *Cell* **58**, 1017–1019.
49. Zopf, D., Bernstein, H. D., Johnson, A. E. & Walter, P. (1990) *EMBO J.* **9**, 4511–4517.
50. Dreyfuss, G., Philipson, L. & Mattaj, I. W. (1988) *J. Cell Biol.* **106**, 1419–1425.
51. Bandziulis, R. J., Swanson, M. S. & Dreyfuss, G. (1989) *Genes Dev.* **3**, 431–437.
52. Berg, O. G., Winter, R. B. & von Hippel, P. H. (1981) *Biochemistry* **20**, 6929–6948.
53. Mazur, S. J. & Record, M. T. (1989) *Biopolymers* **28**, 929–953.
54. von Hippel, P. H. & Berg, O. G. (1989) *J. Biol. Chem.* **264**, 675–678.
55. Kuwajima, K. (1989) *Proteins* **6**, 87–103.
56. Creighton, T. E. (1990) *Biochem. J.* **270**, 1–16.
57. Kim, P. S. & Baldwin, R. L. (1990) *Annu. Rev. Biochem.* **59**, 631–660.
58. Jaenicke, R. (1991) *Biochemistry* **30**, 3147–3161.
59. Kim, J. G., Takeda, Y., Matthews, B. W. & Anderson, W. F. (1987) *J. Mol. Biol.* **196**, 149–158.
60. Fulton, A. B. (1982) *Cell* **30**, 345–347.
61. Lerner, E. A., Lerner, M. R., Janeway, C. A. & Steitz, J. A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2737–2741.
62. Deng, J. S., Takasaki, Y. & Tan, E. M. (1981) *J. Cell Biol.* **91**, 572–577.
63. Fu, X. & Maniatis, T. (1990) *Nature (London)* **343**, 437–441.
64. Spector, D. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 147–151.