Biochemistry

 Volume 29, Number 42 October 23, 1990

Accelerated Publications

Single-Strand DNA Triple-Helix Formation†

Robert Häner and Peter B. Dervan*

Arnold and Mabel Beckman Laboratories of Chemical Synthesis, California Institute of Technology, Pasadena, California 91125

Received July 13, 1990; Revised Manuscript Received August 17, 1990

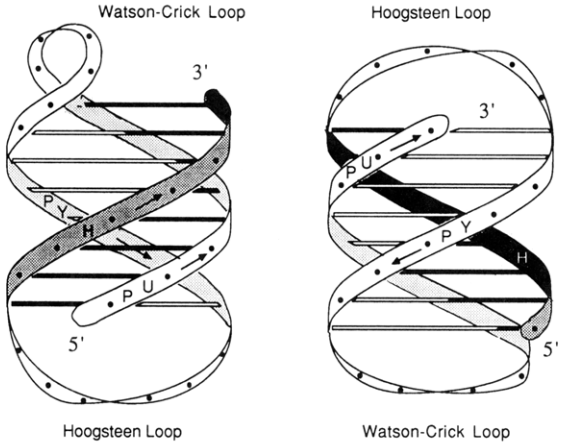
ABSTRACT: Chemical modification studies provide evidence that single-stranded oligodeoxyribonucleotides can form stable intrastrand triple helices. Two oligonucleotides of opposite polarity were synthesized, each composed of a homopurine–homopyrimidine hairpin stem linked to a pyrimidine sequence which is capable of folding back on the hairpin stem and forming specific Hoogsteen hydrogen bonds. Using potassium permanganate as a chemical modification reagent, we have found that two oligodeoxyribonucleotides of sequence composition type 5′-(purine)$_8$(N)$_4$(pyrimidine)$_8$(N)$_6$(pyrimidine)$_8$-3′ and 5′-(pyrimidine)$_8$N$_6$(pyrimidine)$_8$N$_4$(purine)$_8$-3′ undergo dramatic structural changes consistent with intrastrand DNA triple-helix formation induced by lowering the pH or raising the Mg^{2+} concentration. The intrastrand DNA triple helix is sensitive to base mismatches.

Shortly after the discovery of the DNA double helix, Felsenfeld, Davies, and Rich reported that three strands of nucleic acid (RNA) could form a novel polymeric structure in the presence of millimolar concentrations of Mg^{2+} (Felsenfeld et al., 1957). They postulated the existence of a triple helix wherein a pyrimidine strand was bound in the major groove of a right-handed double helix forming specific hydrogen bonds with the Watson–Crick purine strand. Although high-resolution X-ray data for the triple helix has not yet been reported, a considerable body of chemical and spectroscopic data that support the triple-helical model based on T·AT and C+GC triplets exists (Felsenfeld et al., 1957; Lipsett, 1963, 1964; Michelson et al., 1967; Felsenfeld & Miles, 1967; Howard et al., 1964; Morgan & Wells, 1968; Lee et al., 1979; Moser & Dervan, 1987; Praseuth et al., 1988; Rajagopal & Feigon, 1989; de los Santos et al., 1989). In our own work directed at exploring whether the triple-helical structure could be utilized for the function of oligonucleotide-directed sequence-specific recognition of double-helical DNA, we demonstrated that the orientation of the pyrimidine Hoogsteen strand is parallel to the purine Watson–Crick strand (Moser & Dervan, 1987).

Recently, it was proposed that double-stranded DNA can form a triple-helical complex, called H-form DNA (Mirkin et al., 1987). From chemical and enzymatic cleavage protection assays, it appears that double-stranded DNA which contains a homopurine–homopyrimidine sequence with a mirror repeat can form an interstrand triplex by folding part of the pyrimidine strand back on the duplex, leaving a single-stranded purine region (Voloshin et al., 1988; Htun & Dahlberg, 1988; Johnston, 1988; Kohwi & Kohwi-Shigematsu, 1988; Wells et al., 1988; Hanvey et al., 1988). The three-strand and two-strand triple-helical structures have not yet been assigned any definite biological function. In view of the recent discoveries of novel secondary structures formed by single-stranded RNAs such as "pseudoknots" (Henderson et al., 1987; Puglisi et al., 1988; Brierley et al., 1989; Schimmel, 1989) and "hammerhead" structures (Forster & Symons, 1987; Cech, 1987; Uhlenbeck, 1987; Forster et al., 1988), we decided to investigate whether a single strand of DNA or RNA could fold to form a stable triple-helical structure. An *intrastrand* triple helix using T·AT and C+GC base triplets might raise from the folding of a pyrimidine sequence upon a purine–pyrimidine hairpin stem by formation of specific Hoogsteen hydrogen bonds to the purine strand. In this report we describe results obtained in a chemical cleavage–protection assay using potassium permanganate pertaining to the structures of six oligonucleotides of sequence type 5′-(purine)$_8$loop(pyrimidine)$_8$loop(pyrimidine)$_8$-3′ and 5′-(pyrimidine)$_8$loop(pyrimidine)$_8$loop(purine)$_8$-3′ (**1a–c** and **2a–c**).

†Supported by NIH Grant GM35724.

0006-2960/90/0429-9761$02.50/0



1a 5'AGAGAAGATTTTTCTTCTCTTTTTTTTCTCTTCT 3'

1b 5'AGAGAAGATTTTTCTCTTCTTTTTTTTCTCTTCT 3'

1c 5'AGAGAAGATTTTTCTTCTCTTTTTTTTCTTCTCT 3'

2a 5'TCTTCTCTTTTTTTTCTCTTCTTTTTAGAAGAG A 3'

2b 5'TCTTCTCTTTTTTTTCTTCTCTTTTTAGAAGAGA 3'

2c 5'TCTCTTCTTTTTTTTCTCTTCTTTTTAGAAGAGA 3'

FIGURE 1: Models of two different intrastrand triple helices. (Top) The segments are designated as PU (Watson–Crick purine), PY (Watson–Crick pyrimidine), and H (Hoogsteen). The arrows indicate the 5′–3′ direction. (Bottom) The sequences of two series of oligonucleotides investigated that differ in polarity (**1a–c** and **2a–c**).

Experimental Procedures

Materials. Oligonucleotides **1a–c** and **2a–c**, each consisting of 34 nucleotides, were prepared by the automated phosphoramidite method and purified by electrophoresis on 20% denaturing polyacrylamide gels. They were labeled at the 5′-end with T4 polynucleotide kinase (New England Biolabs) and [γ-^{32}P]ATP (Amersham) (Maxam & Gilbert, 1980).

Potassium Permanganate Reactions (Rubin & Schmid, 1980). 5′-End-labeled DNA (10–100 ng) was dissolved in the reaction medium and allowed to equilibrate for 10 min at 24 °C before freshly prepared potassium permanganate (Merck) solution was added. The total reaction volume was 40 μL. The reactions were stopped by the addition of neat allyl alcohol (10 μL). Addition of sonicated calf thymus DNA to a final concentration of 100 μM (bp) was followed by ethanol precipitation and piperidine treatment (90 °C, 30 min) (Maxam & Gilbert, 1980). The residues were suspended in formamide/TBE loading buffer and examined by 20% denaturing polyacrylamide gel electrophoresis. Identical results were obtained when the reaction medium containing the DNA was heated to 90 °C for 5 min and slowly cooled to room temperature or when the DNA was simply mixed and incubated at room temperature.

Results and Discussion

Two different intrastrand triple-helical DNA (or RNA) structures can be considered, 5′-(purine)$_m$loop(pyrimidine)$_m$loop(pyrimidine)$_m$-3′ and 5′-(pyrimidine)$_m$loop(pyrimidine)$_m$loop(purine)$_m$-3′. Oligonucleotides **1a–c** and **2a–c** consist of a purine sequence linked by four pyrimidines ("Watson–Crick loop") to two inverted pyrimidine regions which are connected by six pyrimidine residues ("Hoogsteen loop") (Figure 1). Oligonucleotides **1** have the purine sequence located at the 5′-end, while oligonucleotides **2** have the purine site at the 3′-end. The sequences of **1a** and **2a** differ from each other with respect to their polarity and would give rise to *distinct* triple-helix structures (Figure 1). Oligonucleotides **1b** and **2b** were designed to contain two consecutive mismatched base pairs in the Watson–Crick hairpin stem, whereas **1c** and **2c** would contain two mismatched bases in the Hoogsteen segment of the putative intrastrand triple-helical complex.

Potassium permanganate (Iida & Hayatsu, 1970; Rubin & Schmid, 1980) and osmium tetraoxide (Burton & Riley, 1965; Beer et al., 1966; Friedman & Brown, 1978; Lilley & Palecek, 1984) are commonly used as chemical modification reagents sensitive to secondary nucleic acid structure. Both reagents specifically modify exposed thymidines at the 5,6-positions (Kochetkov & Budovskii, 1972). If the single strand DNA folds into a triple-helical structure, the Hoogsteen pyrimidine strand will likely be protected from modification (Rubin & Schmid, 1980; Lilley & Palecek, 1984) (Figure 1). In the absence of higher order structures such as intramolecular triple helix, the pyrimidine bases in the Hoogsteen strand would be exposed, resulting in chemical modification at those sites. Under the experimental conditions chosen, the Watson–Crick hairpin should be stable and the corresponding pyrimidine bases should be protected. The two loop regions are expected to be exposed and, therefore, subject to chemical modification. Thus, the loop regions serve as internal standards for the sensitivity of the chemical protection assay.

Although qualitatively similar results were obtained with potassium permanganate and osmium tetraoxide when used to investigate oligonucleotides **1a–c** and **2a–c**, we preferred the use of potassium permanganate because the reactions in our hands were more specific and easier to conduct. Oligonucleotides **1a** and **2a** were treated with potassium permanganate over a range of pH values and the cleavage products analyzed on 20% denaturing polyacrylamide gels (Figure 2A,B). Both oligonucleotides were modified at the thymidines located in the loops, regardless of pH. At pH >6.0, the thymidines in the putative Hoogsteen segment are susceptible to chemical modification, suggesting that **1a** and **2a** both consist of a hairpin stem and an unbound pyrimidine segment. At pH <6.0, complete protection of the thymidines in the Hoogsteen segment was observed. These data are in agreement with a structural transition leading to triple-helix formation since the formation of C+GC base triplets likely requires protonation of the cytidines in the third strand. At pH 6.0, there is some, but not complete, protection of the Hoogsteen strand. Both oligonucleotides show additional fine structure in the pH-dependent cleavage pattern. Some thymidines in or adjacent to the Hoogsteen loop are reactive, but modified to a lesser extent at low pH. This seems to indicate that the conformation of this loop allows considerable base stacking at lower pH, which results in the partial protection of these thymidine residues.

The facilitation of triple-helix formation in polynucleotides by polyvalent cations is well-known (Felsenfeld & Miles, 1967). The influence of the magnesium ion concentration on the structures of oligonucleotides **1a** and **2a** was found to be different (Figure 2C,D). Oligonucleotides **1a** and **2a** were treated with potassium permanganate at various pH values in the presence of 1, 10, and 25 mM magnesium chloride concentrations. At pH 6.0, 1 mM Mg^{2+} concentration is sufficient to induce protection of the Hoogsteen pyrimidine

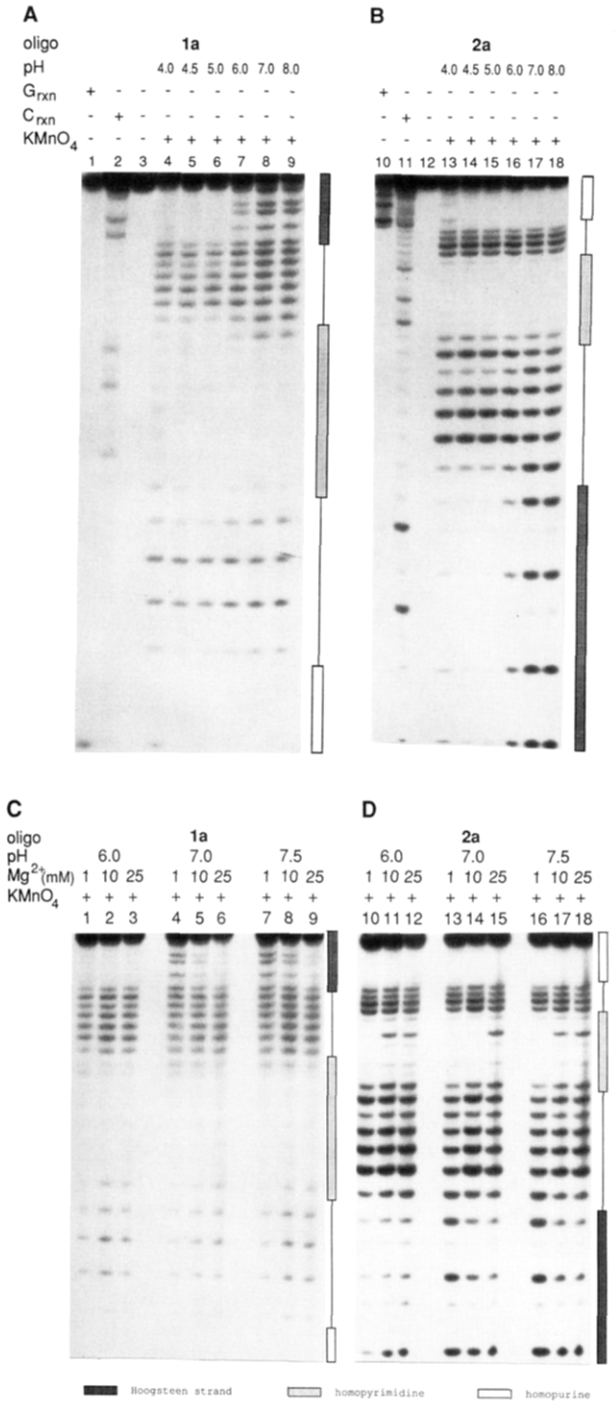


FIGURE 2: Autoradiograms of 20% denaturing polyacrylamide gels obtained after reaction of oligonucleotides **1a** and **2a** with potassium permanganate for 10 min at 24 °C and piperidine treatment. Bars on the right indicate purine and pyrimidine tracts. (A and B) pH dependence of the cleavage pattern for **1a** and **2a**, respectively. Lanes 1 and 10, G-specific chemical cleavage reactions obtained with dimethyl sulfate (Maxam & Gilbert, 1980); lanes 2 and 11, C-specific chemical cleavage reactions obtained with hydroxylamine (Rubin & Schmid, 1980); lanes 3 and 12, intact 5′-end-labeled DNA; lanes 4–9 and 13–18, potassium permanganate reactions with **1a** and **2a**, respectively, at different pH values as indicated. Assay conditions: 5′-end-labeled DNA (100 ng, 250 nM in oligonucleotide), 25 mM NaCl, 25 mM NaOAc (pH 4.0, 4.5, and 5.0) or 10 mM Na_3PO_4 (pH 6.0, 7.0, and 8.0), and 7×10^{-5} M potassium permanganate. Total reaction volume is 40 μL. (C and D) Mg^{2+} concentration dependence of the cleavage pattern for **1a** and **2a** at the pH values indicated. Assay conditions: 10 mM Na_3PO_4 (pH as indicated), 7×10^{-5} M potassium permanganate, and the following $MgCl_2$ concentrations: 1 mM (lanes 1, 4, 7, 10, 13, and 16), 10 mM (lanes 2, 5, 8, 11, 14, and 17), and 25 mM (lanes 3, 6, 9, 12, 15, and 18).

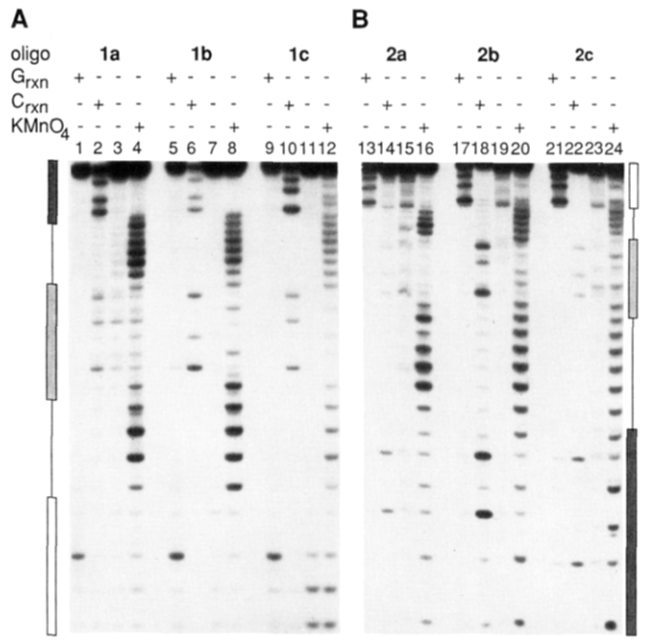


FIGURE 3: Autoradiogram of 20% denaturing polyacrylamide gel showing the cleavage pattern of 5′-end-labeled **1a–c** (A) and **2a–c** (B) obtained after reaction with potassium permanganate. Lanes 1, 5, 9, 13, 17, and 21, G-specific chemical cleavage reactions obtained with dimethyl sulfate (Maxam & Gilbert, 1980); lanes 2, 6, 10, 14, 18, and 22, C-specific chemical cleavage reactions obtained with hydroxylamine (Rubin & Schmid, 1980); lanes 3, 7, 11, 15, 19, and 23, control reactions obtained by treatment as in the cleavage reactions except in the absence of potassium permanganate; lanes 4, 8, 12, 16, 20, and 24, potassium permanganate reactions. Assay conditions: 5′-end-labeled DNA (10–100 ng, 25–250 nM in oligonucleotide), 25 mM NaCl, 25 mM NaOAc [pH 4.5 (A) or pH 5.0 (B)], and 7×10^{-5} M potassium permanganate. Total reaction volume is 40 μL. The reactions were incubated at 24 °C for 10 min (oligonucleotides **1a**, **2a**, **2b**, and **2c**), 7 min (**1b**), and 5 min (**1c**).

strand of oligonucleotide **1a**. Remarkably, at 25 mM Mg^{2+} almost complete protection is observed even at pH 7.5. With oligonucleotide **2a**, the use of 10–25 mM Mg^{2+} renders one thymidine in the Watson–Crick stem sensitive to potassium permanganate at all pH values. The Hoogsteen strand is not protected to an extent comparable to that of the one observed for oligonucleotide **1a**.

The effect of mismatches in the Watson–Crick stem (**1b** and **2b**) and in the Hoogsteen segment (**1c** and **2c**) was examined (Figure 3A). Oligonucleotides **1b** and **1c** are both more reactive to potassium permanganate than **1a**. In order to avoid overdigestion, **1b** and **1c** were incubated for only 7 and 5 min, respectively, compared to 10 min for **1a** under otherwise identical conditions. In addition to those in the loop regions, thymidines in the Watson–Crick pyrimidine strand (**1b**) and in the Hoogsteen strand (**1c**) are reactive (Figure 4A). We examined the influence of mismatches on the structures of oligonucleotides **2a–c** at pH 5.0 (Figure 3B). In this series of compounds, the introduction of mismatches in either the Watson–Crick (**2b**) or the Hoogsteen pyrimidine strand (**2c**) renders most of the thymidines reactive to chemical modification (Figure 4B).

There is always the possibility of intermolecular hybridization of two (or more) strands. Although we cannot rule out intermolecular associations, we believe that by analogy to hairpin formation of synthetic oligonucleotides (Hilbers et al., 1985), intramolecular association should be highly favored at the DNA concentrations used (≤25 nM). During preparation of the manuscript, 2D NMR data were reported that an oligonucleotide of sequence type **1** forms an intramolecular triple helix at millimolar concentrations (Sklerer & Feigon, 1990).

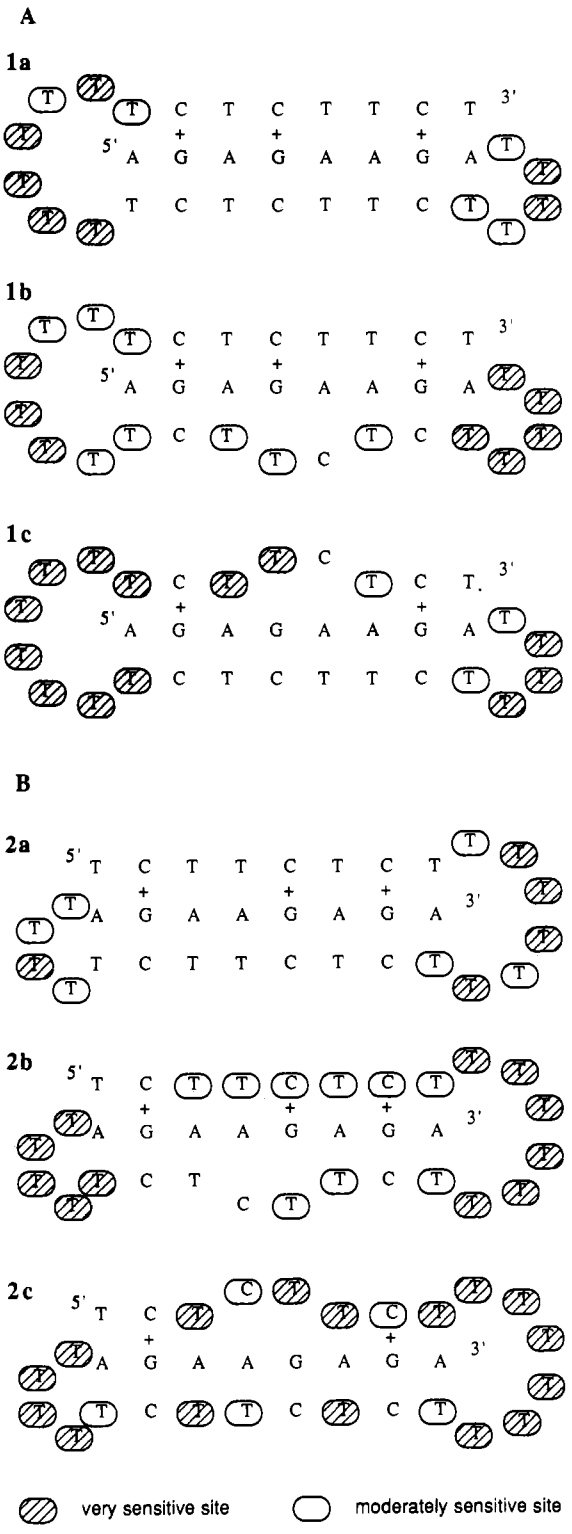


FIGURE 4: Sequence of six 34-base single-stranded DNAs folded into the intramolecular triplex with (a) no mismatches, (b) two mismatches in the Watson–Crick segment, and (c) two mismatches in the Hoogsteen segment. Open circles indicate base positions moderately sensitive to chemical modification. Hatched circles indicate base positions very sensitive to chemical modification. **1a–c** are 5′-(purine)$_8$loop(pyrimidine)$_8$loop(pyrimidine)$_8$-3′ with mismatches indicated. **2a–c** are 5′-(pyrimidine)$_8$loop(pyrimidine)$_8$loop(purine)$_8$-3′ with mismatches indicated.

In conclusion, we find that single-stranded DNA can undergo dramatic structural changes induced by altering the pH or the Mg^{2+} concentration. The results are consistent with a single-stranded DNA adopting an intrastrand triple-helix structure which is sensitive to base mismatches. Of the two orientations, the polarity of oligonucleotide type **1** is preferred for triple-helix formation. This preference may be due to sequence composition effects, and it remains to be seen if this polarity preference is true in all cases. By extension, single-stranded RNA might be expected to adopt similar structures. Though less well understood there is evidence that triple helices containing one pyrimidine and two purine strands exist, presumably facilitated by the formation of G·GC and A·AT triplets (Merck & Thiele, 1978; Broitman et al. 1987; Letaii et al., 1988; Kohwi & Kohwi-Shigematsu, 1988; Cooney et al., 1988). Thus, single-stranded nucleic acids of sequence type purine–loop–purine–loop–pyrimidine containing GGC and AAT triplets might form intrastrand triple-helix structures. Whether these structures exist in vivo or have any biological function is an open question.

ACKNOWLEDGMENTS

We are grateful to the National Institutes of Health for generous support and to Sandoz, Basel, for a postdoctoral fellowship to R.H.

REFERENCES

Beer, M., Stern, S., Carmalt, D., & Mohlhenrich, K. H. (1966) *Biochemistry 5*, 2283–2288.
Brierley, I., Digard, P., & Inglis, S. C. (1989) *Cell 57*, 537–547.
Broitman, S. L., Im, D. D., & Fresco, J. R. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 5120–5124.
Burton, K., & Riley, W. T. (1966) *Biochem. J. 98*, 70–77.
Cech, T. R. (1987) *Science 236*, 1532–1539.
Cooney, M., Czernuszewicz, G., Postel, E. H., Flint, S. J., & Hogan, M. E. (1988) *Science 241*, 456–459.
de los Santos, C., Rosen, M., & Patel, D. (1989) *Biochemistry 28*, 7282.
Felsenfeld, G., & Miles, H. T. (1967) *Annu. Rev. Biochem. 239*, 1256.
Felsenfeld, G., Davies, D. R., & Rich, A. (1957) *J. Am. Chem. Soc. 79*, 2023.
Forster, A. C., & Symons, R. H. (1987) *Cell 49*, 211–220.
Forster, A. C., Davies, C., Sheldon, C. C., Jeffries, A. C., & Symons, R. H. (1988) *Nature 334*, 265–267.
Friedman, T., & Brown, D. M. (1978) *Nucleic Acids Res. 5*, 615–622.
Hanvey, J. C., Shimizu, M., & Wells, R. D. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 6292–6296.
Henderson, E., Hardin, C. C., Walk, S. K., Tinoco, I., Jr., & Blackburn, E. H. (1987) *Cell 51*, 899–908.
Hilbers, C. W., Haasnoot, C. A. G., deBruin, S. H., Joordens, J. J. M., Van Der Marel, G. A., & Van Boom, J. H. (1985) *Biochimie 67*, 685–695.
Howard, F. B., Frazier, J., Lipsett, M. N., & Miles, H. T. (1964) *Biochem. Biophys. Res. Commun. 17*, 93.
Htun, H., & Dahlberg, J. E. (1988) *Science 241*, 1791–1796.
Iida, S., & Hayatsu, H. (1970) *Biochim. Biophys. Acta 213*, 1–13.
Johnston, B. H. (1988) *Science 241*, 1800–1804.
Kochetkov, N. K., & Budovskii, E. I. (1972) *Organic Chemistry of Nucleic Acids*, Part B, Plenum Press, London and New York.
Kohwi, Y., & Kohwi-Shigematsu, T. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 3781–3785.
Lee, J. S., Johnson, D. A., & Morgan, A. R. (1979) *Nucleic Acids Res. 6*, 3073.
Letai, A. G., Palladino, M. A., From, E., Rizzo, V., & Fresco, J. R. (1988) *Biochemistry 27*, 9108–9112.
Lilley, D. M. J., & Palecek, E. (1984) *EMBO J. 3*, 1187–1192.

Lipsett, M. N. (1963) *Biophys. Res. Commun. 11*, 224.
Marck, C., & Thiele, D. (1978) *Nucleic Acids Res. 5*, 1017-1028.
Maxam, A. M., & Gilbert, W. (1980) *Methods Enzymol. 65*, 499-560.
Michelson, A. M., Massoulié, J., & Guschlbauer, W. (1967) *Prog. Nucleic Acid Res. Mol. Biol. 6*, 83.
Mirkin, S. M., Lyamichev, V. I., Drushlyak, K. N., Dobrynin, V. N., Filippov, S. A., & Frank-Kamenetskii, M. D. (1987) *Nature 330*, 495-497.
Morgan, A. R., & Wells, R. D. (1968) *J. Mol. Biol. 37*, 63-80.
Moser, H., & Dervan, P. B. (1987) *Science 238*, 645-650.
Praseuth, D., Perroualt, L., Le Doan, T., Chassignol, M., Thuong, N., & Hélène, C. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 1349-1353.
Puglisi, J D., Wyatt, J. R., & Tinoco, I., Jr. (1988) *Nature 331*, 283-286.
Rajagopal, P., & Feigon, J. (1989) *Nature 239*, 637-640.
Rubin, C. M., & Schmid, C. W. (1980) *Nucleic Acids Res. 8*, 4613-4619.
Schimmel, P. (1989) *Cell 58*, 9-12.
Sklerer, V., & Feigon, J. (1990) *Nature 345*, 836-838.
Uhlenbeck, O. C. (1987) *Nature 328*, 596-600.
Voloshin, O. N., Mirkin, S. M., Lyamichev, V. I., Belotserkovskii, B. P., & Frank-Kamenetskii, M. D. (1988) *Nature 333*, 475-476.
Wells, R. D., Collier, D. A., Hanvey, J. C., Shimizu, M., & Wohlrab, F. (1988) *FASEB J. 2*, 2939-2949.

Reduced Tendency To Form a β Turn in Peptides from the P22 Tailspike Protein Correlates with a Temperature-Sensitive Folding Defect†

Adam N. Stroup‡ and Lila M. Gierasch*,‡,§
Departments of Biochemistry and Pharmacology, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235-9041

Received July 11, 1990; Revised Manuscript Received August 23, 1990

ABSTRACT: A family of mutants of the P22 bacteriophage tailspike protein has been characterized as temperature sensitive for folding (tsf) by King and co-workers [King, J. (1986) *Bio/Technology 4*, 297-303]. There is substantial evidence that the tsf mutations alter the folding pathway but not the stability of the final folded protein. Several point mutations are known to cause the tsf phenotype; most of these occur in regions of the tailspike sequence likely to take up reverse turns. Hence, it has been hypothesized that the correct folding of the P22 tailspike protein requires formation of turns and that the mutations causing tsf phenotypes interfere at this critical stage. We have tested this hypothesis by study of isolated peptides corresponding to a region of the P22 tailspike harboring a tsf mutation. Comparison of the tendencies of wild-type and tsf sequences to adopt turn conformations was achieved by the synthesis of peptides with flanking cysteine residues and the use of a thiol–disulfide exchange assay. We find that the wild-type sequence, either as a decapeptide (Ac-CVKFPGIETC-$CONH_2$) or as a dodecapeptide (Ac-CYVKFPGIETLC-$CONH_2$), has a 3–5-fold greater tendency for its termini to approach closely enough to form the intramolecular disulfide than do the peptide sequences corresponding to the tsf mutant sequences, which have a Gly → Arg substitution (Ac-CVKFPRIETC-$CONH_2$ or Ac-CYVKFPRIETLC-$CONH_2$). A peptide with a D-Arg substituted for the Gly has a slightly higher turn propensity than does the wild type. Together with data from nuclear magnetic resonance analysis of the oxidized peptides, this suggests that a type II β turn is favored by the wild-type sequence. Our results on isolated peptides from the P22 tailspike protein support the model for its folding that includes reverse turn formation as a critical step.

Much research effort is currently directed toward a better understanding of the pathway of protein folding. While well established that amino acid sequence determines the ultimate three-dimensional structure of a native protein, the mechanism by which the linear polymer of amino acids achieves its complex folding pattern remains a puzzle. Most would agree that the ability to predict protein structures from amino acid sequences will be realized only concomitant with an understanding of the protein folding process. Recent evidence that protein folding in vivo often involves factors apart from the nascent polypeptide chain adds another layer of complexity to elucidating the pathway of folding in a cell. It is likely, nonetheless, that the additional factors that facilitate folding in vivo do not alter the basic process but instead ensure that competing processes (e.g., aggregation) are minimized or prevented.

Characterization of folding intermediates has emerged as a key advance in our understanding of the folding pathway. Two recent studies illustrate this approach. Oas and Kim (1988) have modeled an intermediate in the folding of bovine pancreatic trypsin inhibitor (BPTI)[1] by synthesis of two peptide fragments that form a domain likely to be present early in the

† This work was supported by a grant from the National Institutes of Health (GM 27616) and by the Robert A. Welch Foundation.
‡ Department of Biochemistry.
§ Department of Pharmacology.

[1] Abbreviations: Ac, acetyl; BPTI, bovine pancreatic trypsin inhibitor; DMF, dimethylformamide; DIEA, diisopropylethylamine; EDTA, ethylenediaminetetraacetic acid; GSH, reduced glutathione; GSSG, oxidized glutathione; HObt, 1-hydroxybenzotriazole; IAcm, iodoacetamide; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; RP-HPLC, reverse-phase high-performance liquid chromatography; SDS, sodium dodecyl sulfate; TFA, trifluoroacetic acid; Tris, tris(hydroxymethyl)aminomethane; tsf, temperature-sensitive folding; 2-D, two dimensional; WT, wild type.

0006-2960/90/0429-9765$02.50/0