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Protein mimicry of DNA and pathway regulation

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Abstract

The discoveries of DNA mimicry by proteins inspired by Ugi experiments led by Dale Mosbaugh and his colleagues have sparked dramatic insights for our understanding of DNA and protein interactions. Currently only a small number protein mimics of DNA are known or suspected, including Ugi, HI1450, Ocr, TAF1, MfpA, and Dinl. These proteins are structurally diverse, but together they share common themes we define here. These mimics tend to resemble distorted rather than normal B-DNA, possibly to prevent cross-reactions with other DNA metabolizing proteins that should not be inhibited. Side-chain carboxylates of glutamates and aspartates functionally replace phosphates and thereby generate an overall charge pattern resembling the DNA phosphate backbone. Most protein mimics of DNA have strikingly hydrophobic cores that likely stabilize the protein fold despite substantial charge localization and a relatively small internal volume enforced by the restrictions from DNA size. These common characteristics for protein mimicry of DNA should prove useful for future identifications of DNA mimics, which seem likely to be found in bacteriophages, conjugative plasmids, eukaryotic viruses, and transcription machinery. We also suggest approaches to the design of novel DNA mimics to inhibit specific pathways and could be important for basic science applications and for use as therapeutic agents. Moreover, mimicry in general is of critical importance in that it provides an elegant mechanism by which interfaces can be reused to force sequential rather than simultaneous complex formations such as seen in systems involving polar protein assemblies and DNA repair machinery.
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In the late 1980s, Dale Mosbaugh and his colleagues isolated the gene encoding the inhibitor for uracil-DNA glycosylase (Ugi) from the *Bacillus subtilis* bacteriophage PBS2 [1,2]. PBS2 uses uracil in its DNA genome instead of thymine [3], presumably to avoid the action of host restriction enzymes. In general, covalent modifications to DNA are common among bacteriophages, for example the T-even class of bacteriophages use glycosylated 5-hydroxymethylcytosines [4] and phage Mu modifies adenine to generate N^6-(l-acetamindo)adenine [5]. The Ugi inhibitor protein is necessary in the PBS2 bacteriophage life cycle to prevent the activity of host uracil-DNA glycosylase (UDG or UNG) from cleaving uracil bases from the DNA. Ugi has a number of interesting features. First it has an extremely low pI of 3.9, which makes the 9.5 kDa protein run as a 3.5 kDa protein during denaturing electrophoresis [2]. Second, Ugi inhibits UDGs by binding them directly [2]. Structural studies of the UDG/Ugi complex in both our laboratory in collaboration with Dale Mosbaugh and in Lawrence Pearl's laboratory subsequently demonstrated that Ugi was a mimic of double-stranded DNA [6,7]. Thus, Ugi was the first protein identified to function by mimicking the structure of DNA.

Mimicry is common in nature: flies, beetles, and moths mimic bees and wasps; scarlet king snakes resemble the poisonous coral snake. However, proteins that are known to act as structural mimics of RNA and DNA molecules are still quite unusual and informative regarding molecular interactions (Table 1). At the same time Ugi was identified as the first DNA mimic, the EF-Tu ribosomal elongation factor was identified as a tRNA mimic [8], and tRNA mimicry appears to be common to other translation factors as well [9,10]. These tRNA mimics, however, will not be considered here as they have been the subject of a number of excellent recent reviews that, in fact, question whether or not the steric restrictions on their shapes truly indicate that they are tRNA mimics [11,12].

The relative infrequency of known DNA mimics may have several explanations. First, controlling regulation through

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Table 1

Protein	PDBId	Resolution (Å)	Description	Reference
Bacteriophage PBS 1/2 Ugi	lugi	1.55	Uncomplexed	[20]
	2ugi	2.2	Uncomplexed	[20]
	lugh	1.9	Complexed with human UDG	[6]
	ludi	2.7	Complexed with HSV UDG	[7]
	luug	2.4	Complexed with *E. coli* UDG	[20]
	2uug	2.6	Complexed with *E. coli* UDG H187D	[20]
	1lqg	2.9	Complexed with *E. coli* UDG	[78]
	llqm	3.2	Complexed with *E. coli* UDG	[78]
	leui	3.2	Complexed with *E. coli* UDG	[79]
Bacteriophage T7 Ocr	Is7z	1.83	Uncomplexed	[29]
Drosophila TAND-1	ltba	NMR	Complexed with TBP	[44]
H. influenzae HI1450	lnnv	NMR	Uncomplexed	[26]
E. coli DinI	lghh	NMR	Uncomplexed	[54]

protein mimics may simply be less efficient than other potential regulatory mechanisms. Second, effective DNA mimics may be quite toxic as they can potentially interact with many targets. Third, nucleic acid mimics have multiple structural constraints that must be simultaneously satisfied. Fourth, evolution of these mimics may be difficult as suboptimal evolutionary intermediates may be ineffective due to failure to interact with their targets or due to lack of specificity. However, none of these difficulties are necessarily insurmountable, which raises the possibility that many such systems remain to be discovered. Thus, this review, as inspired by our collaboration with Dale Mosbaugh, will describe what the known proteins that mimic nucleic acids illustrate about protein structure, the nucleic acids they are mimicking, and interaction details of the targets they are mimicking.

1. Bacteriophage PBS1 and PBS2 Ugi

The combination of biochemical and structural studies led primarily by Dale Mosbaugh and his colleagues have revealed that Ugi is a remarkable inhibitor of UDG that takes advantage of multiple features of UDG interactions with DNA to obtain both high affinity and high specificity. The 1:1 complex of Ugi with UDGs inactivates UDG from *B. subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Saccharomyces cerevisiae*, rat liver, herpes simplex virus, and humans [2,13,14], but not other DNA glycosylases [14] or general DNA metabolizing enzymes [2]. The UDG–Ugi complex (Fig. 1a) forms via a two-step kinetic process. First, a docking reaction forms an initial, reversible complex. Second, a locking reaction quickly converts the initial complex to an essentially irreversible state that requires protein denaturation to separate [15–17].

Upon recognition of uracil-containing DNA, UDG flips the uracil-containing nucleotide from the DNA base stack and places it within the enzyme active site (Fig. 1b), which involves substantial conformational changes in the DNA [18]. These changes likely take place by multiple steps involving binding, bending, and then nucleotide-flipping of the substrate and concurrent closing down of the enzyme [19]. The closing of the enzyme involves motion of the DNA-binding loops, which is structurally accommodated by a zipping up of the central (three-sheet, previously termed a "β-zipper" [20]. Placement of the extra-helical uracil-containing nucleotide into the UDG active site induces glycosylic bond cleavage through a S_N1 mechanism [21–23].

Ugi is folded into a small five-stranded anti-parallel (three-sheet surrounded on either side by α-helices (Fig. 1a). The packing of these helices generates the central hydrophobic core that stabilizes the protein, which is capable of surviving boiling. Although Ugi is a clear specific mimic of UDG-bound DNA (Fig. 1b), Ugi surprisingly does not mimic the flipped out nucleotide at all, but this was not readily apparent until the first DNA complex was determined [18]. The hydrophobic central core does, however, provide a cavity (Fig. 1c) to accommodate the UDG hydrophobic side chain that displaces the uracil and flips the nucleotide into the enzyme active site (Fig. 1d). This interaction alone is responsible for 250 Å of the 2200 Å of buried surface area in the complex.

The role of carboxylate-containing amino acids (asparatate and glutamate) in Ugi appears to differ somewhat from that of the other DNA mimics detailed in this review. These negatively charged residues do a remarkable job of tracking the positions of the DNA phosphates even for the face of the molecule that does not interact with UDG (compare Fig. 1c and d). This may be important for long-range electostatics acting in complex formation or to improve affinity via electrostatic stabilization. The cluster of negatively charged residues Glu27, Glu30, and Glu30 seems positioned to make long range interactions with UDG Arg268 (human numbering). Moreover, it is intriguing to note that many of the phosphate-like carboxylates on the opposite face of the Ugi inhibitor come in pairs, potentially conserving long-range charge but not precise position (Fig. 1c). But paradoxically, charge seems to be of minimal importance for the face of the molecule that does recognize UDG [20]. In fact, two of the four of the phosphate-binding sites on UDG do not interact with any asparatates or glutamates from Ugi, but rather are recognized by hydrogen-bond acceptors. Where interface carboxylates are used, they are used for geometric reasons not electrostatic reasons. Aspartates and glutamates are the only residues with side chains having multiple hydrogen bond acceptors that are geometrically similar to oxygen atoms in a phosphate group. Geometric use of Ugi carboxylates at the interface mirrors how UDG recognizes DNA. UDG uses hydrogen bond donors, such as serine side chains and backbone amides, to

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