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**Structural analysis of *Escherichia coli* endonuclease VIII and its covalent reaction intermediate**

D. Zharkov (SUNY, Stony Brook), G. Golan (Hebrew Univ. of Jerusalem), R. Gilboa (Hebrew Univ. of Jerusalem), A. Fernandes (SUNY, Stony Brook), S. Gerchman (BNL), J. Kycia (BNL), R. Rieger (SUNY, Stony Brook), A. Grollman (SUNY, Stony Brook) and G. Shoham (Hebrew Univ. of Jerusalem)

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**Introduction:** Endonuclease VIII (Nei) of *E. coli* is a DNA repair enzyme that excises oxidized pyrimidines from DNA. Nei shares with formamidopyrimidine-DNA glycosylase (Fpg) sequence homology and a similar mechanism of action; the latter involves removal of the damaged base followed by two sequential $\beta$-elimination steps. However, Nei differs significantly from Fpg in substrate specificity. We determined the structure of Nei covalently crosslinked to a 13-mer oligodeoxynucleotide duplex at 1.25 Å resolution. The crosslink is derived from a Schiff base intermediate that precedes $\beta$-elimination and is stabilized by reduction with NaBH$_4$. Nei consists of two domains connected by a hinge region, creating a DNA-binding cleft between domains. DNA in the complex is sharply kinked, the deoxyribitol moiety is bound covalently to Pro1 and everted from the duplex into the active site. Amino acids involved in substrate binding and catalysis are identified. Molecular modeling and analysis of amino acid conservation suggest a site for recognition of the damaged base. Based on structural features of the complex and site-directed mutagenesis studies, we propose a catalytic mechanism for Nei.

**Keywords:** DNA repair/endonuclease VIII/oxidative damage/Schiff base/structure