A target protein for nalidixic and oxolinic acids in Escherichia coli, the nalA gene product (Pnal), was purified to homogeneity as judged by gel electrophoresis, using an in vitro complementation assay.The nicking-closing activity is distinct from E. coli omega protein in several properties, including the ability to relax positively supertwisted DNA.DNA gyrase from a strain with a nalA mutation conferring drug resistance (nalA(r)) is 1/100 as sensitive to oxolinic and nalidixic acids with respect to inhibition of supertwisting and induction of the pre-linearization complex.A polypeptide of this molecular weight is uniquely induced by a lambda nalA transducing phage, thereby showing that the purified Pnal is a product of the nalA gene.DNA gyrase preparations and Pnal catalyze a third reaction sensitive to nalidixic and oxolinic acids, the ATP-independent relaxation of supertwister DNA.