DNA Minor-Groove Adducts Formed by a Platinum-Acridine Conjugate Inhibit Association of TATA-Binding Protein with Its Cognate Sequence

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PT-ACRAMTU ([PtCl(en)(ACRAMTU-*S*)]$(NO_3)_2$, en = ethane-1,2-diamine, ACRAMTU = 1-[2-(acridin-9-ylamino)ethyl]-1,3-dimethylthiourea) is a cytotoxic platinum-acridine conjugate that forms adducts with the N3 endocyclic nitrogen of adenine in the DNA's minor groove (Barry, C. G.; et al. (2005) *J. Am. Chem. Soc. 127*, 1160-1169). This unusual observation and the pronounced 5´-TA/TA base-step affinity of the drug (Budiman, M. E.; et al. (2004) *Biochemistry 43*, 8560-8567) have prompted us to investigate the effects of these adducts on DNA minor-groove binding proteins. Here, we used electrophoretic mobility shift assays (EMSA) to study the recognition of a PT-ACRAMTU- modified TATA-box sequence, $(TA)_4$, by TATA-binding protein (TBP). The frequency of PT-ACRAMTU adducts in the minor groove of the TATA box was varied by selective elimination of major-groove and minor-groove potential binding sites in the 24-bp probe sequence (native, probe 1) through incorporation of deaza nucleobases (probes 2–4). The most dramatic effect on TBP binding was observed in a duplex substituted with 7-deaza G and 7-deaza A (probe 3), which reduced binding by max. 73% compared to an unplatinated duplex. In contrast, elimination of A-N3 binding sites (probe 4) had no significant effect on TBP binding, suggesting that minor-groove adducts of PT-ACRAMTU are the cause of inhibition. This notion was further corroborated by efficient platinum-mediated photo-crosslinking (312 nm) of the drug-modified probe 3 to TBP. PT-ACRAMTU appears to be the first platinum-based drug capable of targeting DNA sequences critical for transcription initiation.