Discussion about Stability of Carba-ENA Analogues-Modified AONs in Blood Serum.

The rapid metabolism of the oligonucleotides by a variety of nucleases *in vivo* has to be overcome, if the oligonucleotides were to be utilized as therapeutic or diagnostic reagents. Therefore, blood serum stability (which contain both exo and endonucleases) of AONs **2**, **6**, **10**, **14**, **18**, **22**, **34**, **35** has also been tested. All AONs (^{32}P-labeled at 5′-end) were incubated with human blood serum (male, Type AB) up to 48 h at 21 ^{o}C, and aliquots were taken out at regular time intervals and analyzed by 20% denatured PAGE. The gel pictures obtained by autoradiography are shown in Figure SII 15. Because the alkaline phosphatase in blood serum can remove the 5′-end ^{32}P-label gradually, it is impossible to calculate the accurate digestive rate of each AON from the gel pictures. However, based on the relative contrast amongst the bands on PAGE corresponding to 14mer and 13mer, the qualitative analysis can be made to compare their relative stability in blood serum. In brief, the relative stabilities of all modified AONs in blood serum decrease in the following order: AON **34** ([6′*R*-CH_3-8′*R*-CH_3]-carba-ENA) > AON **35** ([6′*S*-CH_3-8′*R*-CH_3]-carba-ENA) > AON **6** ([8′-CH_3]-carba-ENA) ≈ AON **18** ([8′*R*-NHPAC]-carba-ENA) > AON **26** ([8′*R*-CH_3]-carba-ENA) ≈ AON **14** ([8′*R*-OH]-carba-ENA) > AON **2** (carba-ENA) > AON **10** ([8′*S*-OH]-carba-ENA) > AON **22** ([8′*R*-NH_2]-carba-ENA) > native DNA. Their tendency of stability in blood serum is generally consistent with that found upon treatment with 3′-exonulease, since predominate nuclease activity is of 3′-exonulease in human blood serum. Clearly [8′*R*-NHPAC]-carba-ENA modified AON **18** appears to be more stable than [8′*R*-NH_2]-carba-ENA modified AON **22** and carba-ENA modified AON **2** in blood serum, which contrasts their relative stabilities upon 3′-exonuclease treatment.

Generally, the stabilities of C8′-substitued carba-ENA modified AONs in blood serum are equal or better than C7′-substitued carba-LNA if their substitution type and orientation are the same. For example, carba-ENA (Type **I**) modified AON (stable for 36h) and C8′*R/S*-Me-carba-ENA (Type **II**)