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Further Studies into the Mechanism of Interaction of Negatively Charged Human Serum Albumins with the V3 Loop of the HIV-1 Envelope Protein Gp120.

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Recently, the binding of negatively charged albumins (NCAs), a new class of polyanionic proteins with a potent anti-HIV-1 activity, to certain domains of the envelope glycoprotein gp120 of HIV-1 was demonstrated. Binding of these albumins was predominantly observed at the V3 loop, and is, among others, caused by electrostatic interactions. The present study was performed to determine a possible specific binding site in this V3 loop by testing different peptide fragments of the V3 loop for their interaction with the NCAs. It appeared that binding of NCAs mainly occurred to the tip of the loop (GPGRAF sequence). This sequence is, as compared to the other V3 loop fragments tested, stronger positively charged. However, the amino acid sequence appeared to be important for binding as well, since less positively charged fragments at the C-terminal part of the loop showed an even stronger affinity for the NCAs as compared to the N-terminal part of the loop. Subsequently we studied the interactions of NCAs with a linearilized modulation of the gp120 V3 loop. It has been hypothesized that the V3 loop, after binding of gp120 to the target cell, can possibly be cleaved by cellular proteases, whereafter through conformational changes the actual fusion process is initiated. In the present study cleaving was performed *in vitro* using thrombin and was controlled by SDS-gelelectrophoreses and interaction with V3 specific antibodies. Binding studies of the digested V3 loop with the NCAs showed a complete loss of affinity for the NCAs as compared with the uncleaved loop. Preliminary results indicate even that V3 cleaving *in vitro* can be inhibited by the NCAs. The absence of binding affinity of the NCAs to the opened V3 loop and the high affinity for the closed loop may indicate that the antiviral activity of the NCAs is based not only on shielding of this positively charged gp120 domain from interaction with the cell surface but also prevention of its proteolytic cleavage. Both aspects explains the preferential influence on virus/cell fusion instead of gp120/CD4 binding. We conclude that three factors explain the avid binding of NCAs to gp120: a) electrostatic interactions between the modified albumin and V3 domain, b) the amino acid composition and/or the sequence of the V3 loop, c) the circular conformation of the V3 domain.

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2',3'-Dideoxycytidine 5'-triphosphate analogs as substrate for human DNA polymerases: implication for the mechanism of toxicity

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A number of D- and L-enantiomers of nucleoside analogs have been previously reported to be very potent against HIV or HBV in vitro. At the same time the different cellular toxicity was noticed. The different interaction with cellular DNA polymerases (DPs) can be one of the reasons for their various toxicity.

5'-Triphosphates of separated β-D and β-L-enantiomers of 2',3'-dideoxycytidine (ddC), 2',3'-dideoxy-5-fluorocytidine (FddC), 1,3-dioxalane-cytidine (DOC), 1,3-dioxolane-5-fluoro-cytidine (FDOC) were evaluated as substrates for human DPs alpha, delta, beta and gamma. L-ddCTP proved not to be substrate for any DPs studied; L-FddCTP was not a substrate for replicative DPs and two orders of magnitude less potent inhibitor for DPs gamma and beta in comparison with its D-enantiomer. In contrast all L-dioxolane-analogs were potent inhibitors of cellular DPs. Km of the reaction of incorporation of analogs into DNA chain catalyzed by DP gamma were estimated and was found to be in the following order: ddC<D-FDOC<L-DOC<L-FDOC<<L-FddC. Km of dioxolane-analogs for the reaction catalyzed by DP alpha proved to be 1-2 uM. The interaction of dioxalane analogs with human DPs could be responsible for the expressed cellular toxicity. (Supported by NCI CA44358, NIH AI33655)