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Influenza B/Lee/40 Neuraminidase: X-Ray Structure of the enzyme complexed with 4-guanidino-Neu5Ac2en. N. Y. Chirgadze, J. M. Colacino, K. A. Staschke, K. Briner, W. J. Hornback, J. E. Munroe, R. Loncharich, and W. G. Laver*. Lilly Research Laboratories, Indianapolis, IN. USA. *The Australian National University, Canberra, Australia.

Neuraminidase from influenza B/Lee/40 was crystallized and complexed with the potent and selective influenza neuraminidase inhibitor, 4-guanidino-Neu5Ac2en [1], by soaking the crystal in a concentrated solution of the inhibitor. The enzyme-inhibitor complex was analyzed by X-ray crystallography and experimental data were collected at 2.8 Å resolution with an R-merge of 10.4%. The crystal structure has been refined using a molecular dynamic procedure to yield a current crystallographic R-factor of 16%. The electron density of the inhibitor in the active site is well-defined and interpretation of the electron density distribution reveals an interaction between the C-4 guanidinium moiety of the inhibitor with the glutamic acid at position 117 which lies within a pocket of the active site of the neuraminidase. Similar results have been obtained using influenza A N9 neuraminidase[2]. Computational techniques are being used to analyze the enzyme-inhibitor interaction in terms of H-bond strengths.

[1]von Itzstein M. et al. (1993) *Nature* 363:418-423.
[2]Varghese et al. (1995) *Protein Sci.* 4:1081-1087.

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Resistance of Influenza A and B viruses to 4-Guanidino-Neu5Ac2en. J. M. Colacino[1], K. A. Staschke[1], A. Baxter[1], G. Air[2], A. Bansal[2], E. Garman[3], N. Y. Chirgadze[1], J. Tang[1], W. J. Hornback[1], J. E. Munroe[1], and W. G. Laver[4]. [1]Lilly Research Laboratories, Indianapolis, USA. [2]University of Alabama, Birmingham, USA. [3]University of Oxford, UK. [4]Australian National University, Canberra, AU.

The reassortant influenza viruses, A/NWS-G70c (N9 neuraminidase [NA]) and B/HK/8/73 (HG) (B/Lee/40 NA), were selected for resistance to 4-Guanidino-Neu5Ac2en (4-GuDANA). The NA of resistant viruses was >200-fold more resistant to 4-GuDANA than was the NA of parental viruses. Resistant A and B viruses displayed 5% and 0.5%, respectively, of the parental NA activity yet both were able to undergo multicycle replication in MDCK cells and grow to equal titer in embryonated eggs. The expression by these viruses of NA activity in MDCK cells over a 72 hour period was extremely low relative to that of parental viruses. Sequence analysis revealed a single mutation in the NA gene leading to the change of a conserved Glu 119 (N9 numbering) to Gly for both A and B viruses. Glu 119 lies in a pocket beneath the active site of the enzyme and has been shown to interact with the C-4 guanidinium moiety of 4-GuDANA. The NA from 4-GuDANA^r A/NWS-G70c has been crystallized. Although these crystals grew to only 0.2 mm in the largest dimension, data from low temperature (100K) X-ray diffraction experiments were collected with a merging R-value on intensities of 6.2% to 2.0 Å resolution. These data revealed the absence of the glutamate residue at amino acid position 119. We have been unable to obtain NA crystals of X-ray diffraction quality from the 4-GuDANA^r B virus. In addition to the above viruses, wild-type influenza B/Lee/40 was selected for resistance to 4-GuDANA and the identical nucleotide change leading to the Glu to Gly alteration in the NA was found. Attempts to select a reassortant N2 virus (A/NWS-Tokyo) for resistance to 4-GuDANA have so far been unsuccessful. In preliminary experiments, 4-GuDANA^r A/NWS-G70c was able to induce pyrexia in ferrets indicating that viruses with low-levels of an altered NA retain pathogenicity, at least in this model of infection.