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Structures of VSV glycoprotein ectodomain in both pre- and post- membrane fusion conformation

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Vesicular Stomatitis virus glycoprotein (VSV-G) is involved in both receptor recognition and membrane fusion. Fusion is triggered by low pH-induced structural rearrangements and G can assume at least three different conformations: The native state detected at the viral surface above pH 7, the activated hydrophobic state which interacts with the target membrane as a first step of the fusion process, and the fusion-inactive conformation. There is a pH dependent equilibrium between the different states of G that is shifted toward the inactive state at low pH. Thus, differently from fusogenic glycoproteins from other viral families, the low-pH induced conformational change is reversible. Using limited proteolysis, we have obtained and crystallised a soluble ectodomain of VSV-G in both its neutral and low pH conformations. The structures show that VSV-G is not homologous to any other fusion protein of known structure. Strikingly, VSV-G shares features of both class I and class II fusion proteins, indicative of convergent evolution. The structures also show the extent of the conformational rearrangements and the molecular basis of reversibility. These results invite us to reconsider many questions on the evolution of viruses.

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High-resolution structure of the cofactor regenerator formate dehydrogenase from candida boidinii

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In asymmetric dehydrogenation processes, the necessary but expensive cofactor NADH (reduced nicotinamide adenine dinucleotide) is exhausted. NAD^+ dependent formate dehydrogenase from the yeast *Candida boidinii* (CbFDH) is the preferred cofactor regenerator in industrial biocatalytic enantiomer synthesis. Detailed studies of CbFDH are justified by the considerable biotechnological potential of this enzyme aiming at CbFDH stabilised against applicational degradation. The industrial reaction conditions cause a fast decrease of enzymatic activity resulting in high enzyme consumption. Modification of yeast CbFDH was attempted based on structural modelling on the homolog structure from bacterial FDH from *Pseudomonas sp*.101, (PsFDH) with the aim to circumvent the stability problems. Although the resulting cystein deletion mutants displayed a higher resistance to oxidative degradation, they were of reduced thermal stability and therefore useful for processes in reaction media containing oxidising compounds like metals but not for applications running at temperatures above 45 to 50 C° where denaturation occurs. Crystallisation of CbFDH was unsuccessful for the protein isolated from the native source as well as for the recombinant *wt* protein expressed in *E.coli*. The effective preparation of X-ray quality crystals of CbFDH was only achieved by rational site directed mutagenesis, where surface patches containing residues with large flexible side chains were ideally replaced with smaller amino acids. The mutations were designed by balancing the gain of macromolecular crystallisation enhancement to the preservation of the native structure. Two mutants were tested. Interestingly, the mutated residues are not directly involved in crystal contacts. One mutation stabilised a flexible loop of the protein and the other mediated enhanced crystal contact formation *via* a PEG molecule that was found in a gap occupied by the mutated lysine in the other mutant. We present the high-resolution structure of CbFDH which was obtained from each a C- and an N-terminal single amino acid mutant. We point out the differences in the structure to the bacterial homologue PsFDH from *Pseudomonas sp.*101,. A catalytic mechanism is suggested, which is different from the one proposed for PsFDH. With the crystal structure of CbFDH at hand the attempts to manipulate and optimise the enzyme for industrial cofactor regeneration processes becomes attainable. The structure of the apo CbFDH could serve as a template for modelling structures of mutated enzymes designed to alter the substrate or cofactor specificity or with enhanced catalytic turnover.

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