WWTF Project LS17-069

Diaziridine-FAD: A stable cofactor for biocatalysts and a molecular probe

Abstract

Flavins are key components of many oxidoreductases by serving as prosthetic groups and lending their intricate redox capability to the holoenzyme. Dissociation of FAD leads to an immediate loss of activity and destabilizes the apoenzyme, which compromises the applicability of flavoenzymes in biosensing and biocatalytic applications. Covalent binding prevents dissociation of FAD, but the currently applied genetic or synthetic strategies affect the orientation, catalytic- and redox properties of FAD. Diaziridine functionalization of FAD at the ribityl or adenine group provides universal coupling to the protein scaffold based on specificity and size, without interfering with the redox chemistry of the isoalloxazine group. Photochemical formation of radical species leads to covalent bond formation of the cofactor with amino acids in proximity in correct orientation. Photoaffinity tagging is an established method in medicinal chemistry for covalent receptor-ligand-binding, but its application for linking redox-biocatalysts and cofactors is genuinely novel. This project aims to: (i) synthesize diaziridine functionalized FADs for covalent coupling, (ii) produce native and engineered enzymes used in glucose biosensors for covalent cofactor binding, (iii) study the cofactor occupation of binding-sites, determine stability, enzyme kinetics, redox properties and the performance of the engineered enzymes in biosensors and biocatalysis, and (iv) apply aziridine-FAD as a molecular probe.

Scientific disciplines:
104004 - Chemical biology (80%) | 106002 - Biochemistry (10%) | 209001 - Biocatalysis (10%)

Keywords:
FAD, covalent binding, cofactor, flavoenzymes, glucose biosensor, protein stability, molecular probe

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Further links about the involved persons and regarding the project you can find at
https://archiv.wwtf.at/programmes/life_sciences/LS17-069