Lactococcus lactis Thioredoxin Reductase Is Sensitive to Light Inactivation

Thioredoxin, involved in numerous redox pathways, is maintained in the dithiol state by the nicotinamide adenine dinucleotide phosphate-dependent flavoprotein thioredoxin reductase (TrxR). Here, TrxR from Lactococcus lactis is compared with the well-characterized TrxR from Escherichia coli. The two enzymes belong to the same class of low-molecular weight thioredoxin reductases and display similar $k_{cat}$ values (~25 s$^{-1}$) with their cognate thioredoxin. Remarkably, however, the L. lactis enzyme is inactivated by visible light and furthermore reduces molecular oxygen 10 times faster than E. coli TrxR. The rate of light inactivation under standardized conditions ($\lambda_{max} = 460$ nm and 4°C) was reduced at lowered oxygen concentrations and in the presence of iodide. Inactivation was accompanied by a distinct spectral shift of the flavin adenine dinucleotide (FAD) that remained firmly bound. High-resolution mass spectrometric analysis of heat-extracted FAD from light-damaged TrxR revealed a mass increment of 13.979 Da, relative to that of unmodified FAD, corresponding to the addition of one oxygen atom and the loss of two hydrogen atoms. Tandem mass spectrometry confined the increase in mass of the isoalloxazine ring, and the extracted modified cofactor reacted with dinitrophenyl hydrazine, indicating the presence of an aldehyde. We hypothesize that a methyl group of FAD is oxidized to a formyl group. The significance of this not previously reported oxidation and the exceptionally high rate of oxygen reduction are discussed in relation to other flavin modifications and the possible occurrence of enzymes with similar properties.