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The Lifetime NAD(P)H Fingerprint of Salmonella Infection

Andrea Anzalone[1], Araceli Perez-Lopez[2], Manuela Raffatellu[2], Enrico Gratton[3].
[1]Biomedical engineering, University California Irvine, Irvine, CA, USA, [2]microbiology and molecular genetics, university california irvine, irvine, CA, USA, [3]biomedical engineering, university california irvine, irvine, CA, USA.

NAD(P)H is and endogenous fluorescent coenzyme that has been used as a metabolic biomarker in recent studies using the FLIM technique. Here we describe the NAD(P)H FLIM technique that provides a label-free imaging method to monitor Salmonella enterica infection inside differentiated macrophages. We use FLIM NAD(P)H signature as a marker of bacterial metabolism. NAD(P)H in Salmonella shows a different lifetime fingerprint when the bacteria is grown in a soft agar and when the bacteria infects differentiated macrophages. Specifically, we observed that the free/bound ratio of NAD(P)H in Salmonella changes from a more bound NAD(P)H to a more free state when the bacteria is inside the host cell. During the bacterial infection we also monitored the changes of NAD(P)H surrounding the bacteria inside the macrophage. The NAD(P)H signal around the bacterium when inside the host is interpreted as a marker of the NADPH oxidase enzyme. In contrast to related organisms like Escherichia coli, Salmonella enterica is able to infect macrophages and to escape with high efficiency the attempts of the host cell to kill through the NADPH oxidase enzyme complex. In agreement with this notion, we show that Salmonella changes the state of the surrounding NAD(P)H to a more bound state while in macrophages infected with E. coli we were unable to observe the shift of the NAD(P)H signal surrounding the bacteria. This difference in the NAD(P)H signal around the bacteria corresponds to a reduced activity of the NADPH oxidase around Salmonella, thereby compromising the ability of the enzyme to destroy these types of bacteria.

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