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GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE IS POSTTRANSLATIONALLY MODIFIED BY NAD^+ IN ENTEROHEMORRHAGIC *ESCHERICHIA COLI*

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Several cytoplasmic housekeeping enzymes with no detectable secretion and retention signal are secreted and remain present on the surface of microbial pathogens where they exert functions related to the adhesion and/or virulence of the pathogen. In *Escherichia coli*, extracellular location of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been reported in enterohemorrhagic and enteropathogenic strains. GAPDH can be a target of several covalent modifications including glutathionylation, S-nitrosylation and ADP-ribosylation. Most of these modifications inhibit enzyme activity and may have great physiological consequences. Some of them are linked to stress response or adaptations to new environmental situations.

We have shown that enterohemorragic *Escherichia coli* GAPDH is covalently modified by ^{32}P-NAD^+ in ADP-ribosylation reactions. This activity was assayed in cell extracts and in extracellular isolated proteins. Samples were subjected to polyacrilamyde gel electrophoresis (SDS-PAGE) and visualized by autoradiography. In some experiments, non-radioactive NAD^+, or non-radioactive ADP-ribose were added as putative competitors. Only two proteins appeared to be modified by radiolabeled NAD^+. The band corresponding to the 37KDa protein in SDS-PAGE was excised from silver-stained duplicate gels and identified as GAPDH by mass spectrometry. This result was confirmed by Western blot using anti-GAPDH specific antibodies.

Purified *Escherichia coli* GAPDH is able to promote its own modification by NAD^+. Analysis by 2D gel electrophoresis in immobilized pH 5-8 gradient strips showed that modification by NAD^+ corresponds to the more acidic spots of GAPDH, which represent the less abundant forms of the protein.

To identify the specific amino acid modified by NAD^+, the reaction was carried out with purified GAPDH in the presence of different concentrations of L-cysteine, Lhistidine, and L-arginine. Only free L-cysteine was able to inhibit the reaction, suggesting that NAD^+ modification occurs at cysteine residues.