6. Abstract

The pyridine nucleotides NAD^+ and $NADP^+$ are essential coenzymes in numerous redox reactions in all known organisms. Moreover, NAD^+ and $NADP^+$ are precursors of signal molecules and substrates for covalent protein modifications in higher eukaryotes. When this study was started no molecular data for the essential enzymes of $NAD(P)^+$-synthesis, namely NMNAT and NAD^+-kinase were available. Therefore, molecular and cell biological analyses of $NAD(P)^+$ synthesis and its regulation had not been possible.

In this study, for the first time a proteinchemical and cell biological examination of these two proteins was conducted. The cDNA-sequence of the human NAD^+-kinase was identified. The protein was overexpressed in *E. coli*. The purified enzyme was used to generate antibodies that enabled to the detection of the endogenous NAD^+-kinase in human cells. The enzyme was localised in the cytosol of transfected human cells. Human NAD^+-kinase exibits a molecular mass of approximately 50 kDa and forms a catalytically active homotetramer as demonstrated by gelfiltration experiments. The enzyme is highly specific for its substrates ATP and NAD^+. No phosphorylation of different NAD^+-analogues, among others $NAAD^+$, was observed. Therefore, the NAD^+-kinase that was cloned during this study does nor appear to catalyse the synthesis of the calcium releasing agent $NAADP^+$.

The mRNA encoding NAD^+-kinase was detected in numerous human tissues except muscle. Comparison of the cDNA sequence with genomic sequences and a southern blot analysis led to the localisation of the NAD^+-kinase gene on chromosom 1. The protein sequence of human NAD^+-kinase was compared with other NAD^+-kinases that had been cloned from *M. tuberculosis*, *E. coli* and *S. cervisiae*. 38 conserved residues were identified. These residues are located within a conserved protein domain which has been detected in approximately one hundred so far uncharacterised proteins of different organisms. Among others, there was another human protein containing this domain. This protein potentially represents a second human NAD^+-kinase.

The human NMNAT was also overexpressed in *E. coli*. The purified protein was used to generate antibodies that permitted the visualisation of the enzyme exclusively in nuclei of human cells. The primary structure of NMNAT indicated the presence of potential phosphorylation sites. Indeed a phosphorylation of NMNAT *in vitro* and *in vivo* was detected. A phosphorylation site, Serin 136, was identified by mass spectrometric analyses and was confirmed by mutagenesis studies. This Serin is located in close proximity to the NLS in a surface area of the protein. Serin 136 is part of a consensus sequence that indicates modification by protein kinase C. The influence of the two protein kinase C effectors PMA and BIM on this modification *in vitro* and *in vivo* also supported a phosphorylation by this kinase. The function of the modification is not known yet. No influence on the catalytic activity of NMNAT, the nuclear localisation of the enzyme nor on the interaction with PARP was observed.

These results represent an important progress towards the understanding of $NAD(P)^+$-synthesis and the related energy and signaling processes. For the first time the key enzymes of $NAD(P)^+$-synthesis in eukaryotic cells have become accessible to investigations *in vivo* and *in vitro*.