ABSTRACT

Identification of new human peroxins is an essential step in understanding the biogenesis of peroxisomes. For this purpose three different approaches were followed in this work. At first, a protein potentially homologous to YlPEX23 was identified via sequence comparisons. Immunofluorescence studies showed a mainly cytosolic staining, although parts of HsPEX23 were also localized to the ER. Overexpression of HsPEX23 in human fibroblasts revealed no effect on peroxisomal biogenesis. Antibodies against small HsPEX23 peptides generated within this work allowed detecting overexpressed HsPEX23 in immunofluorescence and western blot experiments. Upon heterologous expression in Saccharomyces cerevisiae, HsPEX23 was detected in peroxisomes by immunofluorescent analysis. During these experiments the formation of peroxisomal clusters could be observed. Therefore, it can be postulated that HsPEX23 has an impact on the biogenesis of peroxisomes in S. cerevisiae.

The purification of human peroxisomes from cell culture was not successful despite the fact that many parameters of the experimental conditions were changed. It was not possible to separate peroxisomes from other cellular organelles by density gradient centrifugation or to obtain significant amounts of peroxisomes allowing for further studies.

Furthermore, a stable, HsPEX14-ProtA-expressing cell line was generated. The analysis of HsPEX14-ProtA in immunofluorescence experiments revealed a distinct peroxisomal localization of the fusion protein. Further experiments showed that the C-terminal Protein A tag did not prevent functionality of HsPEX14. This was proven via complementation studies using a HsPEX14 deficient patient cell line. The fusion protein was able to restore both PTS1- and PTS2-dependend matrix protein import. An existing protocol for complex purification using a protein A tag was adapted to the needs of human cells. Two complexes, which differ in size and peroxin composition, could be separated through size exclusion chromatography.

Finally, the isolated HsPEX14 complex was subjected to analysis by mass spectrometry. In total, 141 proteins eluted with HsPEX14 have been identified. Besides the docking complex peroxins, most of the peroxins of the translocation machinery have been identified. Furthermore, all known peroxins responsible for membrane biogenesis, namely HsPEX3, HsPEX16 and HsPEX19, were found eluted with HsPEX14.

In combination with the results obtained by size exclusion chromatography one can draw the conclusion that there are two different complexes, a matrix protein import complex and a membrane biogenesis complex both including HsPEX14.
In addition, 25 proteins with so far unknown localization and 34 proteins with unidentified function were obtained.

The results obtained in this work suggest for the first time that the human PTS1 receptor (HsPEX5) is ubiquinated at the peroxisomal membrane.