A mass spectrometric approach to investigate cardiolipin metabolism in Barth syndrome
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CHAPTER 10

Summary and Prospects
SUMMARY X-linked cardioskeletal myopathy and neutropenia (Barth syndrome, MIM302060, BTHS) is a disorder with mitochondrial functional impairments and 3-methylglutaconic aciduria that maps to Xq28. The associated tafazzin or TAZ gene has been identified but the encoded proteins have not yet been characterized functionally. A focused search for the biochemical defect in Barth syndrome began with the hypothesis that the gene mutated in Barth syndrome might code for a protein involved in phospholipid metabolism based on the homology of the TAZ gene with acyltransferases. The main focus of this thesis is the study of phospholipid metabolism in Barth syndrome.

This study has given an answer to the question whether Barth syndrome is related to abnormalities in phospholipid metabolism. As shown in this thesis, cardiolipin, a specific phospholipid of mitochondria, is reduced in Barth syndrome compared to the controls in different tissues and cells. To be precise, tetralinoleylcardiolipin, the most abundant cardiolipin in mammals, was markedly decreased while its biosynthesis appeared to be normal indicating a defect in cardiolipin remodeling. The results of our initial attempts to investigate phospholipid metabolism in Barth syndrome illustrated a normal biosynthesis of all major phospholipid classes including that of cardiolipin in contrast to the abnormal remodeling of cardiolipin and its biosynthetic precursor phosphatidylglycerol (chapter 3). These results were obtained using the conventional analytical methods such as Thin Layer Chromatography. In order to investigate the cardiolipin deficiency in Barth syndrome in more details we developed a new method based on online-HPLC-electrospray mass spectrometry.

The application of this method in platelets was found to be a powerful tool for diagnosis of Barth syndrome patients (chapter 4). Using this method a specific tetralinoleyl cardiolipin deficiency in platelets of Barth syndrome patients has been found (chapter 4). In order to answer the question whether the deficiency of tetralinoleyl cardiolipin is specific for Barth syndrome we used the method described in chapter 4 in cultured skin fibroblasts of healthy controls, a group of patients with mitochondrial diseases other than Barth syndrome and a group of patients suffering from Barth syndrome (chapter 5). Although we found that among the diseases used in this study the deficiency of tetralinoleyl cardiolipin was only occurring in Barth syndrome patients, the possibility that this deficiency may also occur among other mitochondrial disorders should not be underestimated.

Since cardiolipin contains four linoleyl acyl groups we investigated the effect of linoleic acid supplementation on phospholipids including cardiolipin in cultured skin fibroblasts. Fibroblasts were cultured under different conditions and phospholipids including cardiolipin were analyzed in a single HPLC run using the same method. Individual phosphatidylglycerol and cardiolipin molecular species and among them molecular species
of other phospholipid families were examined. We found that all phospholipid classes were affected by the supplementation of linoleic acid. To be precise linoleic acid was incorporated in all phospholipids analyzed. The cardiolipin molecular species in culture fibroblast from Barth syndrome patients containing linoleic acid were restored to a near-normal level. The restoration appeared to be time and dose dependent and disappeared when the supplemented culture medium was replaced by normal medium. These results led to the start up of a clinical trial in which patients suffering from Barth syndrome received linoleic acid enriched diet. The first results from this study show a trend of increasing cardiolipin in platelets of Barth syndrome patients. These levels, however, remained below the normal levels (chapter 6).

Next to cardiolipin deficiency we observed accumulation of monolysocardiolipin species and accumulation of linoleate containing phosphatidylethanolamine and phosphatidylcholine in different cells and tissues including heart and muscle tissue of patients suffering from Barth syndrome (chapter 7). The monolysocardiolipin molecular species were composed of mono- or unsaturated fatty acids. This is in contrast with the final fatty acid composition of cardiolipin which contains predominantly linoleic acid. Also the yeast model of Barth syndrome in which the orthologue of the TAZ gene was disrupted showed the same cardiolipin and monolysocardiolipin abnormalities (chapters 8 and 9). Based on these results next to the other available data we formulate the hypothesis that there are two different pathways possible for achieving the final fatty acid composition of CL: a deacylation/ reacylation pathway and a transacylation pathway. The TAZ gene product(s) are most probably involved in the transacylation pathway.

In addition, no correlations were found between the levels of cardiolipin and monolysocardiolipin and mitochondrial function or with the initiation of apoptotic pathway (chapter 7).

**FUTURE PROSPECTS** The work described in this thesis has provided some answers about the function of the TAZ gene product(s), but many questions remain to be answered.

First, the precise mechanism behind the role of TAZ gene product(s) has to be determined. Although the available data are indicative for a role of the TAZ gene product in the remodeling of cardiolipin the exact mechanism of its involvement remains unclear.

Second, it has been shown that cardiolipin is required for the proper function of mitochondria. A comprehensive model that explains how the lack of cardiolipin leads to the manifold mitochondrial dysfunctions in Barth syndrome is not available yet.
Third, it appears that cardiolipin can be remodeled by two different pathways: a deacylation/reacylation pathway and a transacylation pathway. There is, nevertheless, little known about the regulation and the kinetics of the two pathways. Furthermore there is little information with respect to the metabolism of other phospholipids related to cardiolipin metabolism such as phosphatidylglycerol and monolysocardiolipin, and their possible role in Barth syndrome. In addition to this, Barth syndrome is the first functional respiratory chain disorder in which the deficiency is caused by an abnormality in the microenvironment of the inner mitochondrial membrane by the absence of a crucial membrane phospholipid. Hence, studies should be conducted to find out whether there are other mitochondrial disorders caused by a defect in phospholipid metabolism.

An answer to these questions must be found to get a better understanding of the role of cardiolipin abnormality in Barth syndrome and its general importance for the function of mitochondria and hence for the research to find an effective therapy for the patients suffering from this disease. We advocate investigating cardiolipin metabolism in disorders with an unexplained multi-unit respiratory chain deficiency.

One way to get these answers is the use of chemicals with stable isotope labeling in combination with mass spectrometry to unravel at least some of these questions. For example the biosynthesis and remodeling of cardiolipin including its biosynthetic precursors and its remodeling intermediates can be investigated in time under normal and different stress conditions.

To do so, there are some technical aspects concerning the measurement of phospholipids which needs improvement including the mass spectrometric measurement of cardiolipin. The sensitivity and selectivity of the method should be improved in order to carry out the precise quantitative and structural analysis of phospholipids including cardiolipin related compounds, and especially compounds at low concentration levels. More resolution power is needed for a better separation of doubly charged cardiolipin and monolysocardiolipin ions. It is also recommended to synthesize better internal standards for more accurate and precise quantitative measurement of cardiolipin and other phospholipids.