Proposal n. SC1500

Conformational changes involved in the activation of calpain from human erythrocytes

Calpains are intracellular cysteine endopeptidases that combine protease activity with a dependence on Ca^{2+} binding. The major functions of this ubiquitous and widely distributed family of enzymes are related to signal transduction, apoptosis, cytoskeletal remodeling and cell cycle regulation, but they are also implicated in pathophysiological processes, such as cataractogenesis, muscular dystrophies, and Alzheimer's or Parkinson's diseases.

Aim of the experiment was to study the aggregation process of calpain in the presence of calcium ions in solution, in order to characterise the reversible and irreversible mechanisms leading to the activation of this proteinase. The other task of the experiment was to study the structural role of different lipids which are known to have key functional roles in the activation of the proteinase.

In the static set-up, we collected spectra of calpain from human erythrocytes in solution both in the absence and in the presence of different amounts of calcium ions in solution. From these spectra we could evaluate the degree of aggregation of the protein as a function of calcium concentration (fig. 1). From these measurement we could determine that the aggregation process took place in the presence of at least 300 μM excess calcium in solution. This last was the calcium concentration chosen for the kinetic studies.

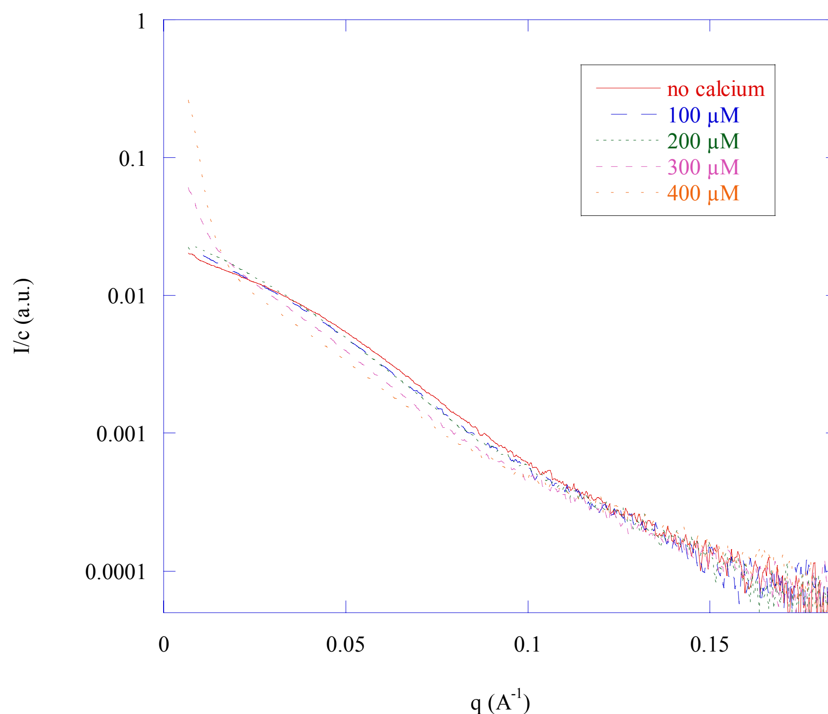

Fig. 1: experimental scattering pattern of calpain in the presence of different amounts of calcium ions in solution.

On the other side, we obtained a very interesting results on the structural role of anionic lipids on calpain. While the addition in solution of 100 μM DPPC had no effect (apart a slight aggregation of the protein), we observed a severe structural change in the presence of 100 μM of other anionic lipids (fig. 2).

The increase of the I(0) value in the scattering pattern indicates the formation of oligomers of the protein. We are actually building an *ab initio* model of the structure of calpain in the presence of different anionic lipids to be compared with the crystal structure of the native proteinase and we are studying the functional effects of different lipids on calpain.

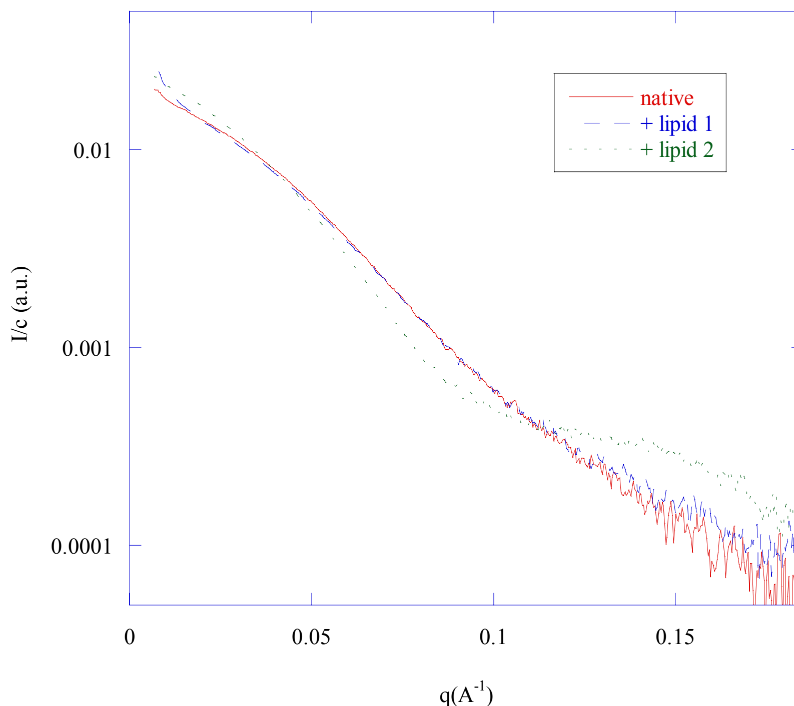

Fig.2: The presence of 100 mM lipid 2 in solution has a dramatic effect in the scattering pattern of calpain from human erythrocytes.

Advantages encountered.

We would like to underline that thanking to the availability of a pump continuously injecting small amounts of protein solution in the capillary, we had the possibility to collect high resolution spectra of the protein solutions in the static set up and no radiation damage was detected in the samples.

Problems encountered

Unfortunately, it has not been possible to perform valuable kinetic measurements to characterise the intermediate states of the calpain during the aggregation process in the presence of calcium.

At first, we had problems with the program controlling the stopped-flow apparatus, which were solved in a few hours by our local contact with some members of the technical staff of the ESRF. Once fixed the problem, we tried to mix the protein solution with calcium, but had constantly bubbles in the capillary, probably formed during the mixing in the reservoirs. Moreover, when some signal was detected, the protein concentration was much lower than the expected one. This observation lead us to conclude that some diffusion (leak) took place in the tubes inside the stopped-flow apparatus and we had no control of the effective volumes injected in the capillary.