ANNUAL REPORT 2005

ACADEMIC YEAR 2004-2005

Scientific publications in 2005
6. MOLECULAR BIOPHYSICS

Professors and Researchers:
Luigi Sportelli
Rosa Bartucci
Rita Guzzi

Postdoc fellows:
Manuela Pantusa
Andrea Stirpe

Undergraduate Students:
Francesco De Simone
Cristina Vecchio

Technical staff:
Bruno De Nardo
Carmine Prete

Collaborators:
D. Marsh (Max Planck Institut for Biophysical Chemistry, Goettingen, Germany)
S.A. Dzuba, D.A. Erilov (Institute for Chemical Kinetics and Combustion, Novosibirsk, Russian Academy of Science and Dept. of Physics, Novosibirsk, Russian Federation)
D. Grasso, C. La Rosa, D. Miliardi (Dipartimento di Chimica, Università di Catania, Italy)
G. W. Cantrters, M. Ph. Verbeet (Gorlaeus Laboratory, University of Leiden, Netherlands)
S. Cannistraro, L. Andolfi (Dept. of Environmental Science, University of Tuscia, Viterbo, Italy)
B. Rizzuti, (Licryl, CNR-INFM, Dept. of Physics, University of Calabria, Italy)
V. Lippolis, A. Garau (Dip. to di Chimica Organica and Analitica, University of Cagliari, Italy)

Introduction

In the year 2005 the research activity has essentially concerned with two main topics in the field of Molecular Biophysics. In particular, the first has concerned self assembled supramolecular lipid structures, their dynamic properties and interaction with active biomolecules, while the second topic has regarded the thermal and spectroscopic properties of Metal-Proteins with oxidase activity. Molecular Dynamics Simulation of a Metal-Protein, Azurin, has also been considered. In the following the main results obtained in each field are reported.

6.1 DYNAMICS PROPERTIES and INTERACTIONS in SELF ASSEMBLED SUPRAMOLECULAR LIPID STRUCTURES

6.1.1 Bipolar Tetraether Lipids: Chain Flexibility and Membrane Polarity Gradients from Spin-Label ESR

Membranes of thermophilic archaea are composed of unique tetraether lipids in which C40, saturated, methyl-branched biphytanyl chains are linked at both ends to polar groups. In this paper membranes composed of bipolar lipids P2 extracted from the acidothermophile archaean Sulfolobus solfataricus are studied. The biophysical basis for the membrane formation and thermal stability is investigated by using electron spin resonance (ESR) of spin-labelled lipids. Spectral anisotropy and isotropic hyperfine couplings are used to determine the chain flexibility and polarity gradients, respectively. For comparison, similar measurements have been carried out on aqueous dispersions of diacyl reference lipid dipalmitoyl phosphatidylcholine and also of diphytanoyl phosphatidylcholine, which has methyl-branched chains. At a given temperature, the bolaform lipid chains are more ordered and less flexible than in normal bilayer membranes. Only at elevated temperatures (80 °C) does the flexibility of the chain environment in tetraether lipid assemblies approach that of fluid bilayer membranes. The height of the hydrophobic barrier formed by a monolayer of archaeabacterial lipids is similar to that in conventional fluid bilayer membranes, and the permeability barrier width is comparable to that formed by a bilayer of C16 lipid chains. At a mole ratio of 1:2, the tetraether P2 lipids mix well with dipalmitoyl phosphatidylcholine lipids and stabilize conventional bilayer membranes. The biological as well as the biotechnological relevance of the results is discussed.

6.1.2 Calorimetric and spin-label ESR studies of PEG:2000-DPPE containing DPPC/lyso-PPC mixtures

The thermotropic behaviour of multibilayers of dipalmitoylphosphatidylcholine (DPPC) containing up to 10 mol% of lyso-palmitoylphosphatidylcholine (lyso-PPC) in the presence and in the absence of low content of the polymer-lipid PEG:2000-DPPE has been studied by differential scanning calorimetry (DSC) and electron spin resonance (ESR) spectroscopy, using the spin probe di-tert-butyl-nitroxide (DTBN). In the absence of polymer-lipids, the main phase transition temperature, \( T_m \), of DPPC is down shifted as function of content of lyso-PPC and the shifts are in the range of \( \Delta T = T - T_m = -0.5 \div -1.5 \) °C. Correspondingly, the cooperative unit, CU, of the transition first
decreases and then levels off with the amount of lyso-PPC mixed with DPPC. In the mixed lipids dispersions the membrane fluidity increases at any temperature. The addition of increasing amount of PEG:2000-DPPE at submicellar concentration, i.e., from the low density mushroom regime to the brash conformational one of PEG:2000 chains, in the lipid matrix composed of DPPC and 10 mol% lyso-PPC do not affect neither the thermotropic phase transition behaviour nor the membrane fluidity and the transition cooperativity of the mixed lipid dispersions.

6.2.3 Time-Resolved Electron Spin Resonance Studies of Spin-Labelled Lipids in Membranes

Pulsed electron spin resonance methods have been applied to study the dynamics and the penetration of water (D$_2$O) in spin-labelled phospholipid membranes. Dipalmitoylphosphatidylecholine membranes, with and without an equimolar amount of cholesterol, have been used, with phosphatidylecholine site-specifically spin-labelled throughout the sn-2 chain.

The librational lipid chain motion in the low-temperature phase of phospholipid bilayers has been revealed by means of primary and stimulated electron spin echo-detected ESR spectra in the sub-nanosecond and microsecond timescale, respectively. The influence of temperature and chain position of labelling on the lipid chain dynamics have been investigated. Three-pulse electron spin echo envelope modulation (ESEEM) spectroscopy is used to determine the transmembrane profiles of the water permeation in the membranes. They display a sigmoidal dependence on position of labelling that is characteristic of polarity profiles. Moreover, a narrow spectral component and a broad one are resolved in the D$_2$O ESEEM spectra of lipid chains spin labelled across the width of bilayer membranes. The two components are assigned by spectral simulations to free and H-bonded intramembrane water molecules, respectively.

6.2 Molecular Dynamic Simulation and Thermal Stability of Metal-Protein with Oxidase Activity

6.2.1 Thermal unfolding of pseudoazurin: calorimetric and spectroscopic studies

The thermal unfolding of pseudoazurin has been investigated by means of differential scanning calorimetry (DSC), optical density, fluorescence and electron paramagnetic resonance spectroscopy. The combination of these experimental techniques has allowed us to gain insight into the modifications of the copper site environment and of the whole protein structure, during the denaturation process.

The thermal transition from the native to the denaturated state results to be irreversible, on the whole, and occurs in the temperature range between 333.0 and 340.5 K, depending on the scan rate and the technique used.

The denaturation pathway of the pseudoazurin can be described in terms of the Lumry-Eyring model: N ⇔ U ⇒ F. The protein reversibly passes from the native (N) state to the unfolded (U) one, whereas the step towards the final (F) state is irreversible and kinetically controlled. This model has been checked by numerical simulation of the calorimetric thermograms. The thermodynamic parameters related to the reversible step (ΔH$_r$ = 498 kJ mol$^{-1}$ and T$_{1/2}$ = 340.8 K) have been obtained by extrapolation of the DSC profile at infinite scan rate. These data together to the ΔC$_p$ value of 8.3 ± 0.9 J K$^{-1}$ mol$^{-1}$, calculated by means two different theoretical methods, lead to a ΔG value of 39.2 kJ mol$^{-1}$ at 298 K.

From the comparison of the data obtained by the different techniques used, it emerges that the thermal denaturation process of holo-pseudoazurin starts with the disruption of the copper active site and the destabilization of the hydrophobic core, and proceeds with the collapse of the whole protein. In addition, according to the EPR findings, the native type-1 copper ion, which is in a distorted tetrahedral configuration in the native state of the protein, shows type-2 copper features after the denaturation.

Finally the role of the copper on the thermal stability of pseudoazurin is also discussed. The unfolding of the apo-form of protein is a reversible process and occurs at 315 K.

6.2.2 Sampling of protein inner motions in molecular dynamics simulations

Molecular dynamics simulation is a powerful technique to investigate the dynamic behavior of a protein in solution. Simulations on the nanosecond time scale are generally considered sufficient to obtain consistent information for physical properties that depend on the motion of protein residues, such as fluctuations, deviations and cross correlated displacements. This concept has been tested on azurin, a protein that represents a good model to study coordinated inner movements because of its small size and inherent stiffness. Dynamical cross-correlation and principal component analysis have been used to assess the convergence of sampling of the protein motions in simulation.

The results show a good correlation between the crystallographic and simulated atomic fluctuations, indicating that the dynamics of azurin, at equilibrium, is similar in the crystal and in solution. Nevertheless, even in this rigid protein the different structural elements (such as beta-strands, hydrophobic patches, alpha-helix) take a relatively long time to coordinate their movements after the simulated annealing. As a consequence, simulations in the range of a few nanoseconds are proven to be insufficient to assess global collective motions of residues.
The information achieved on the nanosecond time scale, though primarily associated to chaotic diffusion, can be used to identify the protein collective degrees of freedom that are more restricted. This has been used to isolate a small, poorly structured region of azurin with high mobility. By filtering out in the data analysis the motions of this region, convergence of sampling for coordinate displacements of residues is obtained for the protein scaffold. Selective filtering of the motions of uninteresting protein regions with high conformational freedom is indicated as a suitable method to investigate the protein inner motions in the time scale commonly sampled in molecular dynamics simulations.
A PUBLICATIONS ON SCIENTIFIC JOURNALS
A.1 Publications on international journals
A.1.1 Publications on international journals printed in 2005


A.1.2 Publications on international journals accepted in 2005


A.2 Publications on national journals
A.2.2 Publications on national journals accepted in 2005


C INVITED PRESENTATIONS
C.1 Invited presentations at international conferences in 2005

1. R. Bartucci, Spin-labelled lipids in membranes: pulsed ESR studies, 4th Workshop on “Protein-Lipid Interactions”, Dubrovnik, Croatia, October 6-9, 2005.

C.2 Invited presentations at national conferences in 2005

1. R. Bartucci, Time-Resolved Electron Spin Resonance Studies of Spin-Labelled Lipids in Membranes,

3. R. Guzzi
   A comparative investigation of the thermal unfolding of pseudoazurin in the holo and apo form,
   XCI Congresso Nazionale SIF, Catania, September 26 – October 1, 2005. Award for the best Oral
   Communication of the Section IVb – Biophysics and Medical Physics.

D PRESENTATIONS AT CONFERENCES
D.1 Presentations at international conferences in 2005

1. R. Bartucci, R. Guzzi, L. Sportelli,
   Time-resolved FT-ESR studies of spin-labelled biosistems,
   5th Workshop on “Molecular Interactions of the Lipid-Protein Interactions”, University of Calabria, September
   10-12-2005.

2. R. Bartucci, R. Guzzi, L. Sportelli,
   Spin-labelled lipids in membranes: pulsed ESR studies,
   4th Workshop on Protein-Lipid Interactions, Dubrovnik, Croatia, October 6-9, 2005.

D.2 Presentations at national conferences in 2005

1. A. Stirpe, R. Guzzi, G.W. Canters and L. Sportelli,
   Thermal unfolding of pseudoazurin: calorimetric and spectroscopic studies
   MMD-Meeting, Italian conference on matter, materials and devices, Genova, June 22-25, 2005 – Italy

2. M. Pantusa, L. Sportelli, R. Bartucci,
   Transfer of stearic acids from Albumin to polymer-grafted lipid containing membranes probed by spin-label
   Electron Spin Resonance,
   MMD– Meeting, Italian conference on matter, materials and devices, Genova, June 22-25, 2005 - Italy

3. B. Rizzuti, L. Sportelli and R. Guzzi,
   Sampling of protein inner motions in molecular dynamics simulations
   MMD– Meeting, Italian conference on matter, materials and devices, Genova, June 22-25, 2005 - Italy

4. R. Guzzi, A. Stirpe, G. W. Canters and L. Sportelli,
   A comparative investigation of the thermal unfolding of pseudoazurin in the holo and apo form,
   SIF-Società Italiana di Fisica, Catania, September 26 – October 1, 2005 - Italy.

5. R. Bartucci, R. Guzzi, L. Sportelli,
   Time-Resolved Electron Spin Resonance studies of spin-labelled lipids in membranes

   Water concentration profiles in membranes measured by ESEEM of spin-labeled lipids,
   MMD– Meeting, Italian conference on matter, materials and devices, Genova, June 22-25, 2005 - Italy

ORGANIZATION OF CONFERENCES

1. Workshop on “Molecular Interaction at the Lipid/Protein Interface”,
   Department of Physics, University of Calabria, September 10-12, 2005.