PML heptads purification and preliminary characterization

F. Antolini1, M. Lo Bello2, M. Helmer2, M. Sette2 and P.G. Pelicci3

1Department of Molecular and Cellular Biology Section of Applied Clinical Biochemistry Via del Giochetto s.n.c. 06100 Perugia - Italy -
2Department of Biology University of Tor Vergata Via della ricerca scientifica s.n.c. 00133 Roma - Italy -
3European Institute of Oncology Via Ripamonti 435 - 20141 Milano - Italy -

The PML gene is involved in the 15;17 chromosomal translocation of acute promyelocytic leukemias (APL). It encodes a nuclear phosphoprotein which is associated, in vitro, with growth suppressor activity. We are currently studying the biochemical and biological properties of the coiled-coil region of the PML protein. It consists of four clusters of hydrophobic aminoacids and is thought to mediate homodimerization and heterodimerization with the PML/RARa mutant protein. The integrity of this region is required for the biological activity of PML and PML/RARa mutant protein. In this report we show the production of PML-heptads as a fusion protein with p-GEX vector. The fused protein was first solubilized, then purified with affinity chromatography. The heptads were obtained after column proteolitic cleavage, then were further analized with FPLC. The FPLC chromatograms, carried out in non denaturing conditions, show the ability of the heptads to elute as a high molecular weight aggregate confirming the evidence that this region is responsible for protein complex formation in "in vivo" experiments. Finally the purified peptide was structurally characterized by means of circular dichroism (CD) measurements. The CD spectra indicate the presence of an a-helix structure. A molecular model of heptad dimer, based on the sequence comparison with other homologue sequences, is also presented.