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Titolo	Interactions between proteins and soil humic substances: evidebces from electrophoeresis, mass spectrometry and nuclear magnetic resonance [Tesi di dottorato]
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Sommario	The destiny of proteins in the environment is still largely unknown. In soil in particular the interaction with soil particles, both inorganic and organic, could be one of the key factors controlling protein fate. Proteins in soil originate from plants, animals and microorganisms and could react, be stabilized or modify their native conformation by the contact with soil colloids. Proteins are very heterogeneous and reactive polymers that in soil environment may share chemical interactions with soil organic matter (SOM), including ionic interactions (both repulsive and attractive), H-bonding, hydrophobic interactions, hydration forces, acid-base interactions and van der Waals forces. Owing to complexity of proteins and SOM, humic substances (HS) particularly, interactions between proteins and HS are considerably less characterized than protein-inorganic phases (i. e. clays) interactions, despite they have been long postulated. In the present PhD project, an investigation about the establishment of interactions between five model proteins (? and ? glucosidase from A. niger, Myoglobin from Hourse muscle, recombinant ovine prion protein and CopH copper binding protein from C. Metallidurans), with distinct chemical and biochemical properties, and purified soil borne humic molecules and a soil has been conducted by complementary

	techniques. (1) Chemosensor, i.e. quartz microbalance, permitted to study the establishing of interaction between partner molecules; (2) protein electrophoresis coupled to (3) mass spectrometry (MS) were used to evaluate possible modifications in protein mobility and identification due to HS presence. (4) Nuclear magnetic resonance (NMR) allowed to prove the establishing of weak interactions between specific chemical regions of the model protein and the used humic molecules. Electrophoretic evidences and MS data, both by soft and less soft ionization methods, are in agreement with the hypothesis that HS are supramolecular associations of relatively low molecular weight organic molecules (LMWOMs), able to interact with specific protein sites, not random, by weak interactions. Protein MS identification resulted affected by the contact with purified HS in term of decrease in coverage % (percentage of protein sequence represented by detected peptides in the dataset) and number of identified peptides (peptides identified by MS and matched to identify the protein). The used MS analytical approach showed that probably non-covalent interactions between proteins and HS-borne could cause the reduction in peptide coverage patterns for all the studied proteins. NMR results supported the previous findings especially by 1H-NMR spectra analysis of relaxation times, line broadening and correlation times. To my knowledge this is the first time this analytical approach is used for studying protein interactions with soil-borne HS, these findings may explain the current limitations in the development of soil proteomics (limited protein identification with the best possible analytical set up). Protein-HS interactions may be one of the factors limiting our knowledge on the protein presence in the environment, representing particularly critical event in the case of pathogenic proteins.
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