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Surface and Solution Structure of Mitochondrial Cytochrome C

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Nonbiogenic amino acids in natural bitumens and synthesis in nature and in experiments

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The so-called "protein" amino acids Asp, Thr, Ser, Glu, Gly, Ala, Val, Ile, Lev, Tyr, Phe, His, Lys, Arg, occasionally Pro, Met have been found in all studied natural solid hydrocarbons (bitumens) and highly carbonaceous substances (shungites). The total content of the amino acids in bitumens regularly increases along the row of their thermal-metamorphic evolution: from 5.7 mg/100g (without NH₃-) in naphthides to 15.96 mg/100g in shungite reaching the highest value (168.3) in highly structured polymeric crystalline fibrous kerite. This regularly suggests that the amino acids resulted from non-biogenic synthesis as a consequence of structural ordering at the molecular and supermolecular levels in hydrocarbons under increasing thermodynamic parameters of their consolidation (solidification, crystallization).

In our experiments we have synthesized amino acids in natural bitumens, irradiating them with a high-energy (5-6 Mev) electron beam, the absorbed dose being 10 and 100 Mrad, and obtained evidence indicating that the amino acids are capable of further assembly giving rise to protein-like and other biopolymeric structures. High radiation doses induce active synthesis of monobasic mono-amino acids (Gly, Ala, Val, Ile, Leu, Ser, Tyr, Thr), monobasic di-amino acids (Arg, Lys) and amino acids with heterocyclic rings (His, Pro). Non-polar aromatic (Phe, Thr), sulphur-bearing (Cys, Met) and dibasic mono-amino acids (Asp, Glu) are less readily synthesized.

Surface and solution structure of mitochondrial cytochrome c

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Redox metalloproteins immobilized on electrodes in the presence of aqueous media are of interest in biomedical applications. In addition, the conformation of redox metalloproteins on inorganic solid surfaces is central to long range electron transfer, enzyme catalysis, and biosensors. In this study, we used Fe K-XANES (X-ray absorption near edge structure) spectroscopy to characterize changes in the vicinity of redox center of mitochondrial cytochrome c (Mcc) in solution and sorbed state as a function of pH and denaturant concentration. Mcc was sorbed to SnO₂ suspensions at pH 2-11 and denaturant (Guanidine-HCl) concentration 1-6 M. The solutions and pastes were analyzed at beamline X-11B at National Synchrotron Light Source (Brookhaven National Lab. NY). All Fe K-XANES data showed three peaks near 7110 (assigned to Fe 1s → Fe 3d), 7125 (Fe 1s → Fe 4s), and 7140 (Fe 1s → Fe 4p) eV respectively. Changes in peak position and intensities in the Fe K-XANES spectra for Mcc solutions suggested subtle conformational changes between pH 5 and 11 and unfolding at pH 2. The structure of Mcc sorbed on SnO₂ differed from its solution structure at all pH's except at pH 9.0 near the isoelectric point of Mcc (pH 9.5) suggesting that Mcc underwent conformational changes during sorption. Guanidine HCl is known to induce unfolding of Mcc. We found systematic changes in the Fe K-XANES spectra of Mcc with an increase in the concentration of denaturant suggesting its gradual unfolding. Surprisingly, there were spectral similarities in the sorbed structure of denatured Mcc at 2, 3 M and Fe sorbed to SnO₂ suggesting that in the sorbed state Mcc unfolded to allow an inner-sphere Fe-O-Sn surface complex. Contrary to our expectations of greater unfolding at higher molarities of denaturant, XANES spectra of sorbed Mcc at 4 and 6 M concentration were similar to the XANES spectra for sorbed Mcc at pH 7 in the absence of denaturant. These results are at variance with our previously obtained electrochemical data of Mcc on SnO₂ electrodes that showed reduction potential similar to the native reduction potential of Mcc suggesting that Mcc maintained native conformation when interacting with SnO₂ electrodes. Hence, our Fe K-XANES data underline the importance of a direct characterization of surface structures of metalloproteins for their appropriate use.