H. Gobind Khorana in the history of protein sequencing

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Before going to British Columbia, Gobind published two papers on the stepwise degradation of peptides, one from N-terminus (H.G.Khorana, Chem, & Ind.(London), 129, (1951)) and another from C-terminus (H.G.Khorana, J.Chem.Soc.(London), 387: 2081, (1952)). P. Edman published stepwise degradation from N-terminus using Phenylisothiocyanate (P. Edman, Acta Chem. Scand. 4: 283 (1950)) one year earlier, but Khorana N-terminus degradation producing free amino acids, could take advantage of Stein Moore amino acid analyzer (S. Moore & W. H. Stein, J. Biol.Chem. 192: 663, (1951)), and lasted until the arrival of HPLC detection of PTH-amino acids.
When I joined Gobind's lab. in Oct. 1976, his interest was back to the proteins after long period of working with the nucleic acids. The idea was, then, to use the phospholipid with built-in photo-activatable probes. After the photolysis leading to the cross-linking with proteins in immediate vicinity, we hoped to obtain the data for understanding the protein topography within the membrane structure. Initially nitrene producing photo-labels were considered, but the careful studies by H. Bayley and J. R. Knowles (Biochem, 17: 2414, 2420(1978)) casted doubt on the usefulness of nitrenes in photolabeling hydrophobic structure, and our group shifted to carbene generating photo-labels. The questions, then, were whether these carbene precursors stable enough in biological systems, and are the photo-crosslinked products amenable to the structural analysis by conventional methods?

The carbene precursor, 2-diazo-3,3,3-trifluoruoro-propionyl group turned out to undergo reduction in the dark by a number of thiols, commonly used as protective agents for proteins (B.B.R.C. 95: 589 (1980)). Consequently, I looked for a protein without requirement to add thiol protectants, and also lacking cysteine. Cytochrome b5 was such protein, and consequently the amino acid sequence of its membrane embedded segments was analyzed (J.Biol.Chem. 255: 1536 (1980)). We managed to get some results for understanding the usefulness of the photo-crosslinking analysis, and the insightful data on the structure of the membrane embedded segments of cytochrome b5 (J.Biol.Chem. 258: 9128, 9136 (1983)).

Along the way, we developed many chemical methodology for the analysis of membrane proteins including the solvent system EtOH : formic acid and Solid-Phase Sequencing technology. I hope to also look back some of the scientific anecdotes that we are worth reflection. (1976 Oct.-1982 May)

Peptide Sequencing from N-terminal

Edman degradation (1950) Khorana degradation (1951)