studies, aimed at identifying molecules that may rescue pathological NPM1 mutants by stabilizing the native-like state.

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P307.

Structurally diverse cyclisation linkers impose different backbone conformations in bicyclic peptides

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Combinatorial repertoires of structurally diverse peptide macrocycles offer a rich source for the development of high-affinity ligands to targets of interest. In this work, we developed linkers for the generation of genetically encoded bicyclic peptides and tested whether the peptides cyclised by them have significant variations in their backbone conformations. Two new cyclisation reagents, both having three thiol-reactive groups, efficiently and selectively cyclised linear peptides containing three cysteines. When the mesitylene linker of the bicyclic peptide PK15, a potent inhibitor of plasma kallikrein (K_i = 2 nM), was replaced by the new linkers, its inhibitory activity dropped by more than a factor 1000, suggesting that the linkers impose different conformations onto the peptide. Indeed, structural analysis by solution-state NMR revealed different NOE constraints in the three bicyclic peptides, indicating that the relatively small linkers at the centre of bicyclic peptides significantly influence the conformations of the peptides. These results demonstrate the prominent structural role of linkers in peptide macrocycles and suggest that applying different cyclisation linkers in a combinatorial fashion could be an attractive means for generating topologically diverse macrocycle libraries.

P308.

Study of Peptide Structural Modifications Induced by Controlled Gamma Ray Irradiation Experiments

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We have initiated[1] an investigation related to the effect of radical species upon structures of some peptide segments. In the proposed experimental protocol, aqueous peptide solution was submitted to gamma ray irradiation in controlled 1-15 kGy doses. The generation of peptide analogues, possibly induced by reactive oxygen species were examined by electrospray triple-quadrupole tandem mass spectrometry (collision induced dissociation approach) and amino acid analysis of crude and/or purified by-products. Noteworthy, the gamma irradiation process induced, regardless of the peptide sequence, a non-linear and progressive degradation of all peptides assayed. Furthermore, these peptides could be classified in some different classes according to their half-life dose. For instance, the vasoactives angiotensin II (AII), Ang (1-7), bradykinin (BK) and some related peptides were more stable than the melanocyte-stimulating hormone α-MSH, Substance P or the BK 's (305-325) B2 receptor fragment (LVYVIVGKRFRKKSREVYQAI). Usually, the most prominent derivatives generated from this experimental protocol revealed that they are likely induced by oxidation process, yielding a variation of +16 Da in their molecular weight. The main source of peptide modifications seems to lie either on the Phe (hydroxyl group insertion at o-, m- or p- positions of its aromatic side chain) or Met oxydation. In the former case, only Phe^8 and not Phe^5 is oxidized in the BK structure whereas Substance P generates an analogue bearing Met-sulfoxide without modifying its $Phe^{7,8}$ residues. Thus, collectively, these findings clearly stress the complexity of factors involved in peptide structural modifications induced by gamma ray-type strong electromagnetic irradiation experiment. An additional target of this approach lies indeed, in the production of unusual peptides for further structure-function investigations. Supported by FAPESP and CNPq.

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P309.

Study of the SL21 peptide structure and its interaction with calmodulin

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SL21 (STOP-like protein with mass of 21 kDa) is a neuronal protein with a calmodulin-binding and microtubule-stabilizing activity. Microtubule binding is inhibited by calmodulin[1,2]. The lack of the STOPs gives rise to defects in synaptic plasticity, as observed in schizophrenia[3].

To study the SL21 protein's structure and its interaction with calmodulin, we have used a 44 residue peptide derived from SL21, containing the microtubule binding motif Mn.

The structure of the peptide has been analyzed using circular dichroism (CD) spectropolarimetry. The CD spectra showed that the peptide's secondary structure consists in a predominantly β-sheets, while the tertiary structure consists in rather folded 3D-structure. The temperature dependence of CD indicated that the secondary structure of the peptide remained very stable between 5 ˚C and 95 ˚C, while the tertiary structure changed at about 35 ˚C.

The interactions between the SL21 peptide and calmodulin have been studied using the isothermal titration calorimetry (ITC). The results showed that