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Amino Acids

Polyamines and Transglutaminases

Direct immobilization of amine oxidases on ethylene-carbon monooxide copolymer

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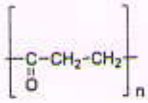
The palladium-catalyzed copolymerization of ethylene and carbon monoxide produces a perfectly alternating polyketone of relatively high crystallinity, to which can be attributed the following formula:



Depending on the polymerization procedure, the ethylene-carbon monoxide copolymer presents some affinity with water. Furthermore, it shows the ability to strongly bind proteins by hydrogen bonds between its keto groups and the amine groups of the amino acids residues of the protein surface and by dipolar interactions. No additional coupling agents or spacer arms are necessary for the immobilization, which is carried out gently, simply mixing end over end the slurry with the protein in diluted neutral phosphate buffer.

The resin was tested with copper amine oxidases isolated from lentil seedling (LSAO) and bovine serum (BSAO) (E.C. 1.4.3.6). In the first case 60 units of enzyme (corresponding to 0.5 mg) have been linked to 100 mg of polymer, giving an apparent LSAO concentration of 1.9 μM in 1.5 ml of slurry. Phosphate buffer 1 M, pH 7.0, containing 1 M NaCl does not remove the protein from the matrix. The immobilized enzyme retains the activity and presents a Km value similar to that shown by the free one. Also specific activity maximum values as a function of temperature and pH are conserved, such as the characteristic biphasic temperature transition.

In the second case, by progressive additions of enzyme, a slurry characterized by an apparent BSAO concentration of 3.6 μM was obtained, without saturating the linking sites of the copolymer.

In conclusion, the matrix appears suitable for enzyme immobilization and purification and for biomedical applications. Biomaterials are important for the controlled delivery of biologically active substances in therapy.

Improvement of bovine serum amine oxidase – spermine toxicity to multidrug-resistant cells by the polyamine oxidase inactivator MDL 72527

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The development of drug resistant tumor cells due to exposure to cytotoxic drugs is one of the major obstacles to successful anticancer chemotherapy. Multidrug resistance (MDR) is associated with several phenotypic alterations. Cells with the MDR phenotype show, among others, a decrease in drug accumulation due to overexpression of P-glycoprotein (P-gp), which acts as an energy dependent pump involved in extrusion of drugs. The purpose of this work was to develop a new strategy to overcome MDR of human cancer cells. By reacting with spermine bovine serum amine oxidase (BSAO) generates cytotoxic products (H_2O_2 and aldehydes). It was shown that cytotoxicity of BSAO/spermine was enhanced by pre-treatment with the polyamine oxidase inhibitor MDL 72527, a compound that had previously been demonstrated to improve the antitumor effect of difluoromethylornithine.

Cell survival experiments were performed on human adenocarcinoma and melanoma cells. These were pre-treated with MDL 72527 at 300 μM, for 24 or 48 hours, and were then exposed to BSAO and 6 μM spermine at 37°C. Cytotoxicity, particularly to MDR cell lines, was significantly higher by the combined treatment, than by BSAO/ spermine alone, even though MDL 72527 did not reduce the number of viable cells under the experimental conditions. An impairment of cell metabolism by this drug was, however, indicated by the formation of numerous vacuoles during the first 24 h of exposure. Their number decreased by 48 h.

The combination of the polyamine oxidase inactivator and the enzymatically formed cytotoxic agents should be of interest in the treatment of multidrug resistant cancers.

The covalent modification of cell proteins by polyamines in cancer prevention

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The elucidation of the role of polyamines remains a challenging problem. Their involvement in the control of events as important as cell proliferation and differentiation is intriguing. Although a simple mechanism of action seems difficult to reconcile with the multiplicity of effects, recent investigations have narrowed the previously proposed hypotheses to a few possibilities. One of the most plausible among these is the regulation of the function of at least some proteins by the posttranslational modification of their structures. In this sense, the specific structural requirements for the activity of polyamines in various organs and cell cultures as modifiers of proteins imply that a common fundamental mechanism underlies their effects. There is considerable evidence to suggest that polyamines may be conjugated to proteins. The studies on the levels of conjugated polyamines in cells have shown a direct correlation between the formation of these compounds and the levels of transglutaminase (TGase) activity. We have been particularly interested in natural agents usually present in the human diet, that suppress cellular growth and induce differentiation involving the posttranslational modification of proteins by structural elements of polyamines. Results indicate that either RA or theophylline induce change in the rate of post-translational modification of protein by polyamines, likely because these drugs increase the activity of TGase and affect the intracellular levels of cAMP. While these data are for the moment based on one histological class of tumor (melanomas), the most important implication of our observation is that it establishes a new way in the screening systems for detecting new molecules able to prevent neoplastic growth.

Ligands as regulators of activity and of stability of transglutaminases

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The activity of tissue tranglutaminase is controlled at the transcriptional level by enzyme induction and at the protein level allosterically by