

STABILIZATION OF ALBUMIN PRODUCT BY N-ACETYL-L-METHIONINE

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Background: Sodium octanoate (Oct) and N-acetyl-L-tryptophan (N-AcTrp) are widely used as stabilizers during pasteurization and storage of albumin (HSA) products. However, N-AcTrp is capable of causing intracerebral disease. Thus, in order to identify a safer alternative stabilizer for albumin products, we screened the properties of a number of amino acids, and found that N-acetyl-L-methionine (N-AcMet) is an excellent candidate.

Results and Discussion: N-AcMet was found to be a superior ROS scavenger. A decrease in the α -helical content of HSA was found to be the smallest in the presence of Oct, without or with N-AcMet. The extent of chemical modification and structural changes of HSA were significantly lesser in the presence N-AcMet than in the presence of N-AcTrp. The anti-oxidant property of chloramine-T treated HSA was best preserved by N-AcMet. N-AcMet is also superior in preserving the normal pharmacokinetics of HSA. Thus, N-AcMet is superior to N-AcTrp in protecting albumin preparations against oxidation. Moreover, in order to investigate the mechanism responsible for the protective effect of N-AcMet from the structural-chemical basis, x-ray crystallographic analysis of the HSA-Oct-N-AcMet complex was performed. The results revealed that the binding site of N-AcMet is located at subdomain IIA that corresponds to Site I, whereas the high-affinity binding site of N-AcTrp on HSA is located at subdomain IIIA (Site II). Interestingly, Oct is found to bind to the inner cavity, with a lower binding affinity, and N-AcMet is bound to the outer-cleft of drug Site I. Oct and N-AcMet are found to bind close to each other. Furthermore, the side chain of N-AcMet that is susceptible to oxidation is exposed to the solvent, suggesting that N-AcMet may have an antioxidant function. On the other hand, Oct increases the thermal stability of the complex by occupying the larger intra-cavity of sub-domain IIIA with a high binding affinity. These limited data suggest that N-AcMet is a useful formulation additive for albumin products such as functionalized HSA and HSA fusion proteins.