BS3 as a potential specific tool for the reticulation of membrane proteins

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Introduction

BS3 (Bis Sulfosuccinimidyl suberate) creates covalent bonds between proteins and is believed to act as a cross-linker for proteins in solutions and cell membranes. In the present work, we sought to determine how BS3 reacted with a purified protein (bovine serum albumin, BSA) in a solution, as well with a given protein (serotonin receptor 5-HT7) in a complex biological matrix.

Materiels and methods

In order to know the optimum ratio between concentrations of BS3 (and BSA), a given quantity of BSA (300µg in 30µL of Ringer's solution) was added to increasing volumes of a 50 mM BS3 solution, the final volume of the medium was adjusted to 100µL. We analyzed [BS3]/[BSA] from 11 to 178. The reaction took place for 30 min at 4°C under agitation until it was stopped by the addition of 10µL of 1M glycine. Aliquots of 10µl of samples were denatured, separated by polyacrylamide gel-SDS electrophoresis, and electro-transferred onto a PVDF membrane. Afterwards, the PVDF was stained with red Ponceau and de-stained with distilled water.

Results and discussion

The decreased intensity of BSA or 5-HT7 bands, when BS3 was present in the medium, indicated a modification of the BSA target protein by BS3. BS3 modified BSA and lead to an additional 79 kDa band, mainly seen when the [BS3]/[BSA] ratio was between 11 and 44. This 12 kDa increase in mass may correspond to the fixation of 18 molecules of BS3 on the 67 kDa BSA. However, no cross-linking between molecules of BSA occurred, as no band corresponding to twice the mass of the BSA protein (134 kDa) was observed. This may be due to the fact that the molecules of BSA were too far away from each other to be reticulated by BS3 in the solution. Also the reaction was ran at 4°C, which does not recapitulate the natural biological environment; the low temperature was used so as to inhibit protease activity.

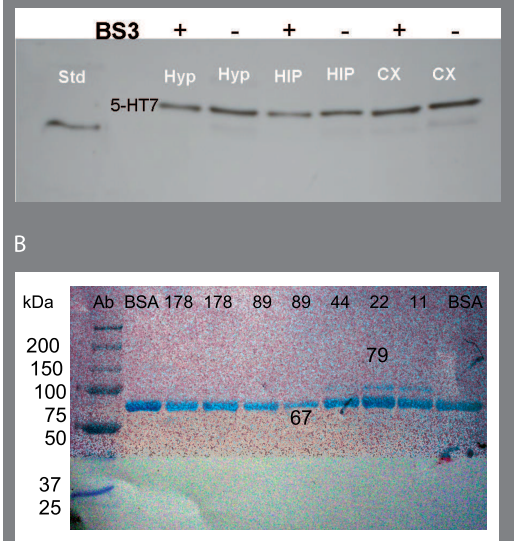

Figure 1: Effect of various BS3 concentrations. A: Effect of BS3 on the total fraction of 5- HT7 receptor. Brain homogenate incubated with (+) or without (-) BS3.
B: Effect of BS3 on standard BSA (0.45 10-4 mol/L). BS3 concentration: 0.5 to 8 10-3 mol/L giving [BS3]/[BSA] from 11 to 178 as shown at the top of each lane. Blue bands: All Blue Standards ; masses are indicated on the left.

Conclusions

As BS3 cannot cross the membrane of the cell, it can only react with the extracellular part of trans-membrane proteins, leaving intracellular proteins unaffected. Thus, the membrane 5-HT7 receptor should be reticulated with closely located membrane proteins leading to high mass complexes; in contrast, the intra-cellular (internalized) 5-HT7 receptor will keep its original molecular mass. Consequently, these 2 forms of 5-HT7 receptor will be separated by an SDS-PAGE gel, allowing for a determination of the percentage of distribution between membrane and intracellular fractions. Thus, it will be possible to study the dynamics of the internalisation of the 5-HT7 receptor and thus to better determine the implication of this protein in terms of both biological and pharmacological phenomena.

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