16ª Reunião Anual da Sociedade Brasileira de Bioquímica, Caxambú, MG, Brazil, 22-25 Apr, 1987.

abstract only

B11

EVALUATION OF THE CHLORAMINE T STOICHIOMETRIC IODINATION OF hGH, hLH, hPRL, hTSH, hCALCITONIN AND BSA REFERENCE PREPARATION

MT Ribela, FA Araujo, EK Gimbo, I Schwarz, L Morganti, V Borghi and P Bartolini
Divisão de Radiobiologia, IPEN-CNEN/São Paulo

The stoichiometric iodination which uses limiting amounts of Chloramine T (Ch.T), described by J.Roth (Meth Enzymol 1975;37:223) is currently the most widely used technique for the 125-I-labelling of protein hormones. Compared to the classical technique described by WM Hunter it has some clear advantages: a)it uses a much lower Ch.T concentration; b)it does not use metabisulfite or other reductants; c)it allows a better control of iodine incorporation with consequent prediction of the tracer specific activity. Its unique disadvantage is, in our opinion, the great discrepancy existing between iodine incorporation calculated via TCA precipitation or via the more accurate analysis of the reaction mixture (ARM). This discrepancy is mainly due to incontrollable bovine serum albumin (BSA) iodination, always occurring in this reaction, especially in the absence of a reductant. We decided therefore to introduce the stoichiometric iodination checking, and possibly trying to improve its accuracy. Its performance was studied for five different hormones (hGH,hLH,hPRL,hTSH,hCalcitonin) and for a reference protein (BSA). The TCA precipitation technique was improved by the following modifications: (i)use of small glass balls derived from home made fused capillaries,which allowed replicate testing and avoided pipetting; (ii)use of a blank (I incorporation before Ch.T addition) and addition of carrier KI in the TCA reaction in order to detect and eliminate as much as possible 125-I non-specific bindings; (iii)counting of the washed out capillaries so to check the affinity of the labelled hormone for glass.

The following results were obtained: 1)instead of the fixed Ch.T amount (50µg) normally used, the optimal amounts varied between 1.5 and 6.0 µg. 2)KI addition to the TCA reaction clearly improved our blanks (from 10.9±7.2 % to 3.1±1.9 %) and consequently the accuracy and precision of the test; 3)the tracer affinity for glass surfaces varied from -5% (BSA) to 33-40% (hLH & hCT) still being highly aleatory even for the same hormone; 4)TCA precipitation versus ARM presented values, for iodine incorporation, whose interpolated ratio (slope of the curve) was 1.33 (r=0.855). This will probably improve only with the elimination of incontrollable BSA iodination; 5)BSA iodination, used as a quality control of the labelling reagents (125-I included), presented a yield of 30.7 %, which is perfectly acceptable into the pool of values previously obtained with the classical technique: 32.0±6.7 % for n=8.