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Conjugates of beta-lactam antibiotics with carrier proteins: Synthesis, properties and bioanalytical application

Berlina A N, Sanarova D D, Bartosh AV, Zherdev AV and **Dzantiev B B**
A N Bach Institute of Biochemistry, Research Center of Biotechnology of RAS,Russia

Hapten-protein conjugates are a key reagent in a variety of bioanalytical systems, providing registered competitive binding of bioreceptor molecules (antibodies, aptamers, etc.) with native molecules of detectable low molecular weight compounds and their conjugates. Upon conjugation, the choice of synthesys protocol affects the affinity of interactions with bioreceptors and, accordingly, parameters of the developed assays. The study was directed to the comparison of techniques for conjugation of proteins with beta-lactam antibiotics, which are important analytes to be measured for estimation of effectiveness of antibioticotherapy and providing safety of consumer products. Bovine serum albumin (BSA) was used as a carrier protein. The haptens were ampicillin (Amp), which has a free primary amino group in the structure, and penicillin G (PenG) without amino group. Conjugation was performed by activated ester method using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysulfosuccinimide (NHSS). Four synthesys techniques were implemented, differing in quantity of interacting reagents and activators: (i) 31 nmol BSA, 72 μmol Amp, 0.3 mmol EDC; (ii) 31 nmol BSA, 72 μmol Amp, 0.15 mmol NHSS; (iii) 310 nmol BSA, 16 μmol PenG, 0.3 mmol EDC; (iv) 310 nmol BSA, 16 μmol PenG. The works on conjugates obtaining and assessing their binding to antibodies were financially supported by the Ministry of Science and Higher Education of the Russian Federation (agreement No. 14.613.21.0061 of 17.07.2017, the unique project identifier RFMEFI61317X0061). Enzyme immunoassay and immunochromatography demonstrated the maximum affinity of the conjugate obtained by the first method. With its use, the detection limit of ampicillin was 80 ng/mL.

Biography

Anna N Berlina has completed her PhD from AN Bach Institute of Biochemistry, Russian Academy of Sciences. Her interests laid in the area of analytical chemistry, nanotechnology and alternative labelling in immunoassay.

berlina.anna@gmail.com

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