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Conjugation of Antibody to Ferritin by Means of *p,p'*-Difluoro-*m,m'*- dinitrodiphenylsulphone¹

Ferritin conjugates of specific antibodies prepared with bifunctional reagents such as diisocyanates have proved useful as tools in immuno-electron microscopy (1, 2). In using *m*-xylylene diisocyanate or toluene-2,4-diisocyanate, a two-step procedure had to be employed, as the antibody was inactivated by the diisocyanates. This involved prior reaction of ferritin with the reagent, followed by a second step in which the antibody was added to the reaction mixture (1, 3, 4). Using *p,p'*-difluoro-*m,m'*-dinitrodiphenylsulphone (FNPS) (5, 6), we have been able to conjugate ferritin to rabbit globulins containing antibodies to bovine serum albumin (BSA) (7), in a one-step procedure with little loss of precipitating capacity of the antibody. FNPS has been shown to react primarily with ϵ -amino groups of lysine and to a lesser extent with the tyrosyl groups (8, 9) in proteins. In preliminary studies the following procedure proved adequate to yield an active antibody-ferritin conjugate. A reaction mixture of 200 mg. globulins containing rabbit anti-BSA (prepared by fractionation with 50% ammonium sulfate), 2 ml. normal saline, 2 ml. 4% sodium carbonate, 4 ml. Pentex ferritin (340 mg.), and 5 mg.

FNPS dissolved in 1 ml. acetone, was stirred continuously at 4°C. for 24 hr., dialysed extensively against normal saline and centrifuged to remove a small amount of precipitate. A control preparation with antibody and ferritin was similarly set up with 1 ml. of acetone. (Reaction of antibody with FNPS under the same conditions without ferritin, also yielded an active derivative.) The control and test preparations were compared by physical-chemical and immunochemical techniques. The presence of an active antibody-ferritin conjugate in the test solution was established by the following criteria. (1) Cellulose acetate paper electrophoretic studies (barbitone buffer, pH 8.6) revealed the presence of two spots in the control and three in the test solution. The spots in the control and two of the three spots of the test solution corresponded to ferritin and globulin. The third spot of the test solution which was also the most intensely colored and easily recognizable as containing ferritin by its brown color even prior to staining, moved with a mobility intermediate to that of ferritin and globulin. Ferritin, rabbit globulin, and their respective reaction products with FNPS served as additional controls in the paper electrophoretic studies. (2) Precipitin studies (10) made against the antigen, BSA, with the test and control preparations showed in both cases zones of antibody excess, equivalence, and antigen excess. After three washings with saline, the precipitate obtained in the test preparation was dark brown while that of the control was white. Iron analysis (11) revealed the presence of the metal in the former precipitate and none in the latter. That the precipitates were specific and of immune type was also established by the absence of precipitation either from the test preparation or the control by the addition of an unrelated protein such as β -lactoglobulin or normal rabbit serum. Immuno-electron microscopic studies with ferritin conjugates prepared as described here, and evidence for the suitability of FNPS as a general reagent for conjugation of two proteins, will be published elsewhere.

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