Communications to the Editor

Chem. Pharm. Bull. 33(1) 448–450 (1985)

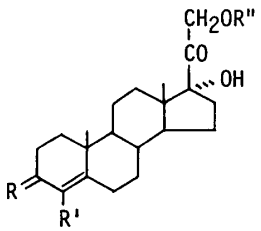
AN ENZYME IMMUNOASSAY METHOD WITH A MONOCLONAL ANTIBODY FOR THE DETERMINATION OF 11-DEOXYCORTISOL

Hiroshi Hosoda,[a] Sakiko Tamura,[a] Norihiro Kobayashi,[a]
Toshio Nambara,*[,a] Jun-ichi Sawada,[b]
and Tadao Terao[b]
Pharmaceutical Institute, Tohoku University,[a] Aobayama, Sendai 980, Japan and National Institute of Hygienic Sciences,[b] 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158, Japan

An enzyme immunoassay method using a monoclonal antibody for the determination of 11-deoxycortisol is described. The anti-11-deoxycortisol antibody was produced in ascites by inoculating antibody-secreting cells into BALB/c mice. The N-succinimidyl esters of carboxylated steroids were treated with β-galactosidase to give enzyme-labeled antigens. The bound and free fractions were separated by a double antibody-protein A method. It was found that the monoclonal antibody had a high affinity for a homologous enzyme-labeled antigen prepared from 4-(2-carboxyethylthio)-11-deoxycortisol and the binding was inhibited by the analyte 11-deoxycortisol, resulting in a satisfactory assay sensitivity.

KEYWORDS—— enzyme immunoassay; 11-deoxycortisol; monoclonal antibody; β-galactosidase; 11-deoxycortisol-β-galactosidase conjugate; protein A

Immunoassays of 11-deoxycortisol in human plasma are useful in the metyrapone test,[1] an assessment of pituitary-adrenal reserve. We have recently prepared a monoclonal antibody to 11-deoxycortisol by the cell hybridization method:[2] the hybridoma line producing the antibody was derived from fusion of P3-NS1/1-Ag4-1 myeloma cells with spleen cells of BALB/c mice immunized with 4-(2-carboxyethylthio)-11-deoxycortisol (I) linked to bovine serum albumin. The anti-11-deoxycortisol antibody (IgG_1) has shown a satisfactory specificity in the radioimmunoassay procedure using tritium as a tracer. It is desirable to develop a specific and sensitive enzyme immuno-



11-deoxycortisol: R=O, R'=R"=H
I: R=O, R'=$S(CH_2)_2COOH$, R"=H
II: R=$NOCH_2COOH$, R'=R"=H
III: R=O, R'=H, R"=$CO(CH_2)_2COOH$

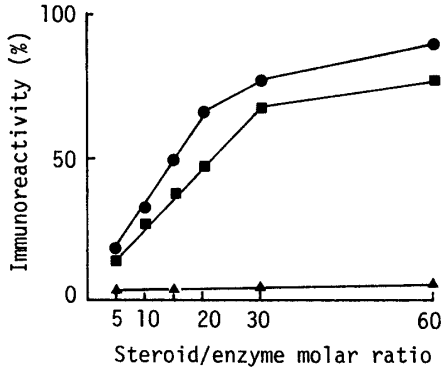


Fig. 1. Immunoreactivities of β-Galactosidase-Labeled Antigens Prepared from I (●), II (■), and III (▲) with the Monoclonal Antibody

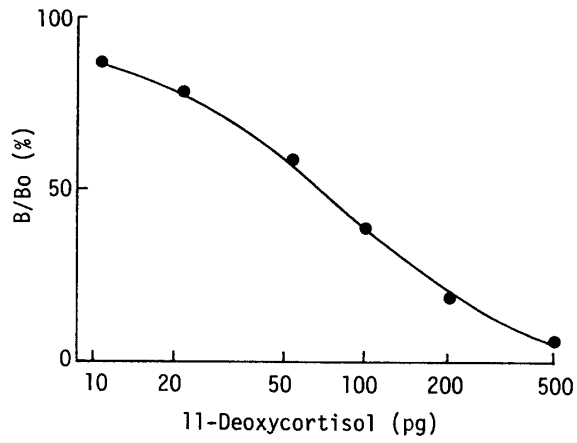


Fig. 2. A Dose-Response Curve for 11-Deoxycortisol Enzyme Immunoassay

assay system using the monoclonal antibody. For this purpose the antibody must first be capable of binding an appropriate enzyme-labeled antigen, and then the immunoreaction between the antigen and antibody molecules should be efficiently responsive to a minimum amount of the analyte 11-deoxycortisol. These specifications depend on the specificity of the antibody and its relative binding affinities for the analyte and enzyme-labeled antigen. We report here preliminary successful results on enzyme immunoassay procedures with the monoclonal antibody for the determination of 11-deoxycortisol.

The monoclonal antibody used in this work was produced in ascites by inoculating the hybrid cells i.p. into pristane-treated BALB/c mice. The steroid derivatives used for enzyme labeling were thioether (I), and two typical haptenic compounds, 11-deoxycortisol 3-(O-carboxymethyl)oxime (II) and 11-deoxycortisol 21-hemisuccinate (III). The N-succinimidyl esters of these carboxylated steroids were treated with β-galactosidase at various molar ratios of steroid to enzyme, ranging from 5 to 60, to give enzyme-labeled antigens.[3)]

Enzyme immunoassay was carried out in 0.05 M phosphate buffer containing 0.1% gelatin, 0.9% NaCl, and 0.1% NaN_3 (assay buffer) as follows: the enzyme-labeled antigen (0.1 μg, 0.1 ml) and diluted antibody (0.1 ml) in the buffer were added to a series of standard 11-deoxycortisol solutions (0.1 ml), and the mixture was incubated at 4°C for 16 h. Rabbit anti-mouse immunoglobulin antiserum diluted 1:500 (0.1 ml) and 5% IgGsorb (a suspension of Staphylococcus aureus containing protein A) (0.1 ml) were added to the incubation mixture, and the suspension was vortex-mixed, then allowed to stand at 4°C for 2 h. After addition of assay buffer (1.5 ml), the resulting solution was centrifuged at 3000 rpm for 20 min, and the supernatant was removed. The precipitate was diluted with assay buffer (1 ml) containing 0.2% $MgCl_2$ and 0.7% 2-mercaptoethanol, vortex-mixed, and preincubated at 37°C for 3 min. o-Nitrophenyl β-D-galactopyranoside (0.06%, 1 ml) in the buffer was

added to the resulting solution, and the mixture was incubated for 1 h. The reaction was terminated by addition of 1 M Na_2CO_3 (2 ml). The absorbance was then measured at 420 nm.

The binding abilities of a fixed amount of the labeled antigens were investigated at 1:10000 dilution of the monoclonal anti-11-deoxycortisol antibody, and the results are shown in Fig. 1. In the homologous assay system[4] using label I, the immunoreactivity increased with increasing molar ratio and satisfactory binding abilities were obtained with molar ratios higher than 10. A lower reactivity was observed in the case of label II. On the other hand, the monoclonal antibody did not significantly bind label III. These results indicate that the antibody recognizes the 21-hydroxyl group of 11-deoxycortisol, and to a lesser extent the functional groups near the C-4 position. In other words, the antigen-binding site of the antibody is complementary to and covers the steroid portion remote from the position used for attachment of the carrier in the preparation of the immunogen.

A typical dose-response curve obtained with the homologous assay system employing a 1:100000 dilution of the anti-steroid antibody and the label prepared at a molar ratio of 20 is shown in Fig. 2. The sensitivity of the assay is comparable to that of the radioimmunoassay,[2] showing that this antibody has weak, if any, binding affinity for the bridge portion[5] between the enzyme and steroid in the homologous labeled antigen I.

Thus, the present findings suggest that the monoclonal antibody may be useful in the development of a practical enzyme immunoassay for plasma 11-deoxycortisol. In this work, the bound and free enzyme-labeled antigens were separated by a double antibody-protein A method. When applied to the assay system using horseradish peroxidase-labeled antigens, however, the separation method did not give satisfactory results owing to loss of enzymic activity. In this regard, triple or solid-phase antibody methods may be advantageous. This is under investigation. Development of other immunoassay systems using ^{125}I-radioligands or fluorescent and chemiluminescent labels should be possible, if the position C-4 of the steroid molecule is employed as a conjugation site; the use of the position C-3 seems also to be worthy of such examination. Application of the present assay system as well as the radioimmunoassay method[2] to the metyrapone test on patients with Cushing's syndrome are being conducted.

ACKNOWLEDGEMENT This work was supported in part by a grant from the Ministry of Education, Science and Culture, and from the Science and Technology Agency of Japan.

REFERENCES

1) G.W. Liddle, H.L. Estep, J.W. Kendall, Jr., W.C. Williams, Jr., and A.W. Townes, J. Clin. Endocrinol. Metab., 19, 875 (1959).
2) H. Hosoda, N. Kobayashi, T. Nambara, J. Sawada, and T. Terao, Chem. Pharm. Bull., 32, 381 (1984).
3) H. Hosoda, Y. Sakai, H. Yoshida, and T. Nambara, Chem. Pharm. Bull., 27, 2147 (1979).
4) B.K. Van Weemen and A.H.W.M. Schuurs, Immunochemistry, 12, 667 (1975).
5) H. Hosoda, N. Kobayashi, and T. Nambara, Chem. Pharm. Bull., 31, 953 (1983).

(Received November 20, 1984)