## Communications to the Editor

[Chem. Pharm. Bull. 33(2) 902—904 (1985)]

# THE BRIDGE LENGTH EFFECT ON SENSITIVITY IN STEROID ENZYME IMMUNOASSAY

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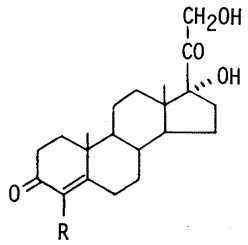
The effect of the bridge heterologous combination between antiserum and enzyme-labeled antigen on sensitivity in 11-deoxycortisol enzyme immunoassay has been investigated. The sensitivity of an assay system using a β-galactosidase-labeled antigen prepared from an 11-deoxycortisol derivative having a carboxymethylthio moiety as a bridge at C-4 was compared with one with an N-(carboxymethyl)carbamoylmethylthio moiety at C-4. The former assay proved to be more sensitive than the latter. This indicates that the bridge length is an important factor influencing the sensitivity of hapten enzyme immunoassay.

KEYWORDS—— enzyme immunoassay; hapten; 11-deoxycortisol; β-galactosidase; anti-11-deoxycortisol antiserum; β-galactosidase-labeled 11-deoxycortisol; immunoassay sensitivity

Development of a steroid enzyme immunoassay system having high sensitivity and specificity comparable to those of radioimmunoassay is not always easy. Steroid antibodies are elicited in animals by immunization with the steroid molecule linked via a "chemical bridge" to a carrier protein. It is reasonably well substantiated that the specificity of antiserum is significantly influenced by the position on the steroid molecule used for conjugation to the carrier,[1] and that the combination between antiserum and enzyme-labeled antigen is an important factor determining the assay sensitivity.[2] We reported previously that a "bridge" heterologous system rather than "site" heterology is preferable as regards assay specificity[3] and the use of enzyme-labeled antigen prepared from a hapten having a bridge shorter than that used for the antibody preparation increases sensitivity.[4,5] The latter proposal is based on




11-deoxycortisol: R=H
CMT: R=$SCH_2COOH$
CMTG: R=$SCH_2CONHCH_2COOH$
CET: R=$S(CH_2)_2COOH$
HST: R=$S(CH_2)_2OCO(CH_2)_2COOH$

the results obtained with various enzyme immunoassay systems for cortisol[4] and 11-deoxycortisol.[5] This paper presents further evidence for the bridge length effect on sensitivity in steroid enzyme immunoassay.

In the present work, sensitivities obtainable with 11-deoxycortisol assay systems using β-galactosidase-labeled antigens CMT and CMTG in combination with anti-11-deoxycortisol antisera CMT, CET and HST were compared. The bridge length of CMTG is longer than that of CMT by the glycine unit, and condensation of the carboxyl groups of these haptenic derivatives with the amino group of a lysine residue in β-galactosidase results in a close analogy in their bridge structures, thus yielding a set of bridges of varying length. The antisera used were those elicited previously in rabbits by immunization with the conjugate of the corresponding hapten CMT, CET, or HST with bovine serum albumin.[6]

The enzyme labeling was carried out by the N-succinimidyl ester method,[7] in which the steroid/enzyme molar ratio of 4 was used. Immunoassay was done with three homologous and five heterologous combinations, using 100 ng of the labeled antigen per tube. The bound and free enzyme-labeled antigens were separated by a double antibody method, and the enzymic activity of the immune precipitate was determined colorimetrically with o-nitrophenyl β-D-galactopyranoside as a substrate.

Table I. Inhibition of Bound Enzymic Activity of Enzyme-Labeled Antigens by 200 pg of 11-Deoxycortisol in the Assays Using Antisera CET and HST

| Labeled antigen | Antiserum CET | | Antiserum HST | |
|---|---|---|---|---|
| | Dilution | Inhibition (%) | Dilution | Inhibition (%) |
| Homologous | 1:4000 | 30 | 1:8000 | 35 |
| CMT | 1:4000 | 56 | 1:2000 | 74 |
| CMTG | 1:4000 | 37 | 1:2000 | 57 |

Sensitivity obtainable with all the assay systems was tested by examining the inhibition of enzymic activity caused by the addition of 200 pg of 11-deoxycortisol. The assays were assessed in terms of the absorbance of $B_0$ obtained upon 1 h enzymic reaction; the criterion that the optical density should be at least 0.1 was employed. The results obtained with the antisera CET and HST are listed in Table I. In each assay system, even when higher dilutions of the anti-steroid antiserum were used, no significant increases in the sensitivity were observed. Comparison of the cases of homology and heterology showed that sensitivity was increased in the heterologous systems. It is obvious that the enzyme-labeled CMT is more effective than the labeled CMTG in increasing the sensitivity. A comparison of two combinations between the antiserum CMT and the labeled antigens CMT and CMTG gave findings compatible with these results (data not shown).

The present results indicate that the bridge length is an important factor influencing the sensitivity of hapten enzyme immunoassay and the use of a shorter bridge for enzyme labeling results in an increase in sensitivity. We have explained this effect as being the result of the steric interaction between antibody and labeled enzyme.[4] Further studies are being conducted to determine whether the bridge length effect depends on the enzyme used.

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

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(Received January 14, 1985)