New Method for Peroxidase-Protein Conjugation

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Conventional methods of conjugation of proteins with peroxidase (HRP) utilize bifunctional reagents. The products have been the result of random coupling and the efficiency was about 5% at maximum. By the improved method the carbohydrate moiety of fluorodinitrobenzene-blocked HRP was oxidized with sodium periodate to form aldehyde groups. The peroxidase-aldehyde was then bound to free amino groups of proteins unidirectionally at high efficiencies.

The current procedure for the preparation of HRP labeled IgG is as follows:

Five mg of HRP is dissolved in 1.0 ml of freshly made 0.3 M sodium bicarbonate, pH 8.1–8.3. To block α- and ε-aminogroups of HRP is added 0.1 ml of 1% fluorodinitrobenzene in ethanol, and mixed gently for 1 hour at room temperature. The carbohydrate moiety of HRP is oxidized with 1 ml of 0.08 M sodium periodate in distilled water, mixing gently for 30 minutes at room temperature. The oxidation is stopped by addition of 1 ml of 0.16 M ethylene glycol in distilled water. After 1 hour reaction at room temperature, the solution is dialyzed against 0.01 M sodium carbonate buffer, pH 9.5, overnight at 4°C. These reaction results in HRP-aldehyde. HRP-aldehyde is reacted with 5 mg of protein by mixing gently for 2–3 hours at room temperature. Then 5 mg of sodium borohydride is added and left at 4°C, 3 hours to overnight to stabilize the conjugate. After dialysis against PBS overnight at 4°C, the sample is applied to on 85 × 1.5 cm column of Sephadex G-200 equilibrated in PBS. The first peak is pooled and examined for enzymatic and immunological activities.

With the above procedure, approximately 70% of HRP should be coupled with IgG and approximately 99% of the IgG should be labeled with HRP. No significant losses of either IgG immunologic or HRP enzymatic activities occur.

The possibility is suggested that various proteinaceous antigens labeled with HRP could be used in immunoassay system in place of radiolabeled antigens, and that polypeptide hormones coupled with HRP without significant loss of biological activity could be utilized in the study on the hormone receptors.

Fundamental Study of Immunohistochemistry

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1) The properties of conjugates prepared by different methods

Several methods have been reported on the technique to prepare the conjugated