

Effect of protein on glycerol color development during the assay of lipoprotein lipase

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SUMMARY The inhibition by proteins of color development in the estimation of glycerol during assay of lipoprotein lipase is shown to be due to the binding of formaldehyde to protein in a form preventing subsequent color formation.

FOR THE purpose of assaying the lipolytic activity of lipoprotein lipase preparations, Korn (1, 2) modified the glycerol determination procedure developed by Lambert and Neish (3) in order to be able to measure the amount of glycerol liberated as the enzyme acted upon triglyceride substrates. The method depends upon the oxidation of glycerol to formaldehyde with excess periodate, the reduction of the periodate and iodate to iodide with excess sodium arsenite, and the development of a purple color from formaldehyde with the chromotropic acid reagent developed by MacFadyen (4). Included in the lipase assay mixture during incubation, oxidation and color development are the enzyme; 10% bovine serum albumin (Armour's fraction V) which acts as a fatty acid acceptor; chylomicrons or "activated" triglycerides (2% triglyceride plus serum proteins) which act as substrates; and 0.5 M $(\text{NH}_4)_2\text{SO}_4$ which provides an "activating cation." In our hands this method has produced extremely variable results.

While studying clearing factor levels in lipemic serum with Lipomul as an added substrate, Cleland and Iacono (5) noticed a 25% loss in glycerol recovery when the serum proteins were present in the assay mixture. Korn (2) suggested that when large amounts of protein are

present in the assay mixture, deproteinization may be necessary. Suehiro and Nakanishi (6) showed that adding increasing amounts of serum to the assay mixture inhibited color development, with the inhibition being removed by deproteinization. Kern, Steinmann, and Sanders (7) and Stern, Iacono, and Mueller (8) routinely deproteinized their assay mixtures before color development but did not explain why this procedure should prevent color inhibition.

When increasing amounts of bovine serum albumin (up to the amount usually present in the assay mixture) are added to a glycerol solution equivalent in concentration to that present in the assay mixture, an inhibition up to 27% in the development of color from glycerol occurs (Fig. 1). This inhibition occurs to the same extent whether or not an equivalent amount of albumin is present in the reagent blank. Color inhibition also occurs if glycerol is added in increasing amounts to a standard amount of albumin. Under these conditions there is not a constant inhibition of color development which would allow for correction by a standard curve. If, however, the assay mixture is deproteinized with 10% trichloroacetic acid just prior to the oxidation of glycerol to formaldehyde with periodate, there is no inhibition of color development (Fig. 1). With this added step the

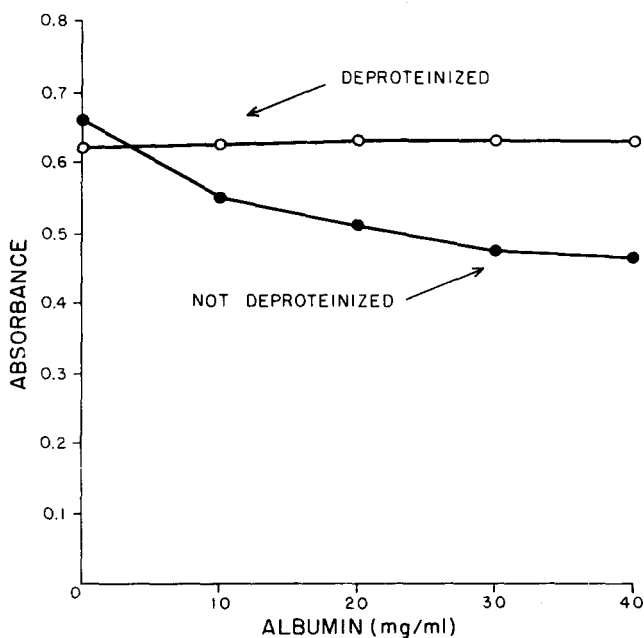


FIG. 1. Effect of added albumin on glycerol color development. Various amounts of 10% bovine serum albumin (pH 8.5) were added to 0.014 μ moles of glycerol and brought to a final volume of 1 ml. For deproteinization the glycerol and albumin were increased to maintain the same final concentration, 2 ml of cold 10% TCA was added at 4°, and the solution was allowed to stand for 10 min. 0.2 ml of each solution was assayed for glycerol by the method of Korn (2). Each point represents three determinations.

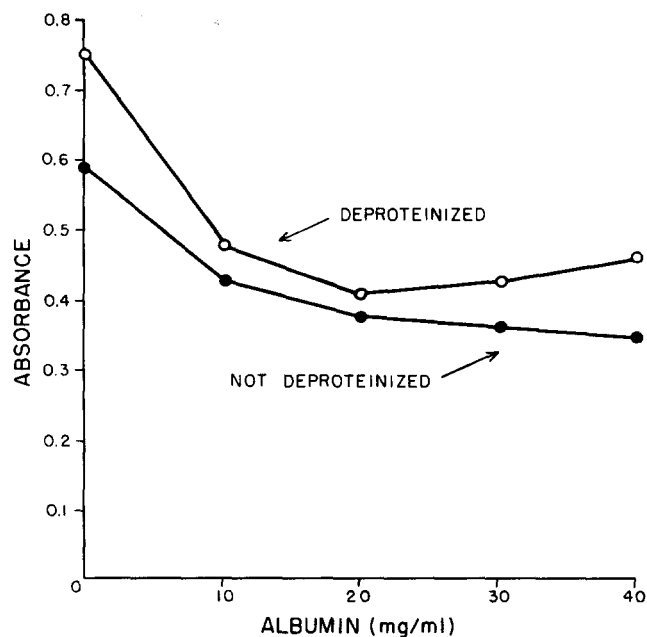


FIG. 2. Effect of added albumin on formaldehyde color development. Various amounts of 10% bovine serum albumin (pH 8.5) were added to 0.1 ml of a formaldehyde solution (which was approximately at the same concentration as that yielded by the glycerol in Fig. 1) to make a final volume of 1 ml. These calculations were based on Korn's assumption (2) that each mole of glycerol yields 2 moles of formaldehyde upon periodate oxidation. The procedures for concentration adjustments, deproteinization, and assay for formaldehyde were the same as those listed in Fig. 1. Each point represents three determinations.

glycerol in the assay mixture can be recovered with an accuracy of $\pm 1\%$.

When the bovine serum albumin is added in increasing amounts to a solution of formaldehyde present at about the same concentration as that found in the assay mixture (Fig. 2), a color inhibition up to 40% is obtained. In order to rule out any effect which the sulfuric acid used in the method might have in terms of denaturing the albumin, the formaldehyde also was added to an albumin solution containing the proper amount of sulfuric acid. The presence of sulfuric acid in the albumin solution prior to the addition of formaldehyde did not affect the degree of color inhibition.

The color inhibition with formaldehyde remains essentially unchanged when deproteinization is carried out just prior to color development (Fig. 2). This would indicate that the formaldehyde is bound to protein (9), is unavailable for color development when bound to protein, and is removed from the solution with deproteinization. Therefore, during the assay of lipoprotein lipase activity using the glycerol method (1, 2), deproteinization of the assay mixture just prior to the oxidation of glycerol to formaldehyde is essential to prevent the loss of formaldehyde by protein binding.

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