ANTIBODY STUDIES WITH THE MULTIPLE ENZYMES OF HORSE LIVER ALCOHOL DEHYDROGENASE II.[1]

Regina Pietruszko[2] and Howard J. Ringold[3]
Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts
Nathan Kaplan[4] and Johannes Everse
Brandeis University
Waltham, Massachusetts

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In the prededing communication (Pietruszko, et al., 1968) it was shown that the component C_1 of horse-liver alcohol dehydrogenase (ADH), which contains an "alcohol" site and a "steroid" site, cross-reacts efficiently with antiserum prepared against the major ADH component C_3, which contains two "alcohol" sites. The fact that both the steroid and alcohol activity of C_1 were removed at an identical equivalence point strongly reaffirms the conclusion that C_1 is a hybrid enzyme containing a subunit identical with that of C_3. In this paper we present data showing that antiserum formed against C_1 also precipitates steroid and alcohol activity at the same equivalence point and cross-reacts efficiently with C_3.

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[2]Present Address: Department of Biochemistry, Nobel Medical Institute, Stockholm, Sweden.

[3]Present Address: Syntex Research, Palo Alto, California.

[4]Present Address: Department of Biochemistry, University of California at San Diego, San Diego, California.

Rabbit antiserum to C_1, which was active in complement fixation at a dilution of 1:3,200, was prepared as described in the experimental section. This antiserum gave a single

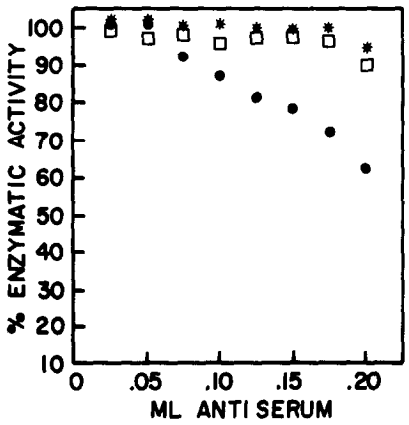

Figure 1: Enzymatic activity of C_1 and C_3 in the presence of increasing amounts of antiserum to C_1. High concentration of enzyme and antiserum; assay carried out in the presence of precipitate.

● - 5βDHT as substrate, enzyme C_1
* - cyclohexanone as substrate, enzyme C_1
□ - cyclohexanone as substrate, enzyme C_3

Ouchterlony diffusion band with C_1 but no visible band with C_3. Figure 1 illustrates the enzymatic activity of C_1 and C_3 when a relatively concentrated enzyme solution (90 µg/7.25 ml) was titrated with neat antiserum solution and the precipitated complex was not removed. While only a minimal inhibitory effect was noted with either enzyme C_1 or C_3 when cyclohexanone was the substrate, the inhibition of steroid reduction in the presence of C_1 was significant (ca. 40%) at 0.2 ml of antiserum. Higher concentration of antiserum could not be used because the heavy precipitate interfered with the spectrophotometric determination. In Figure 2 it may be seen that inhibition was much greater when the antiserum-enzyme precipitate was removed by centrifugation.

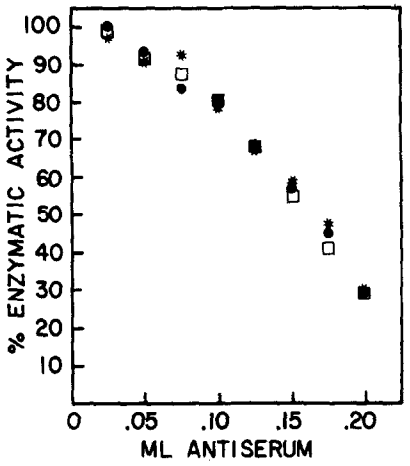

Figure 2: Enzymatic activity of C_1 and C_3 in the presence of increasing amounts of antiserum to C_1. High concentration of enzyme and antiserum; precipitate removed.

• - 5βDHT as substrate, enzyme C_1
* - cyclohexanone as substrate, enzyme C_1
□ - cyclohexanone as substrate, enzyme C_3

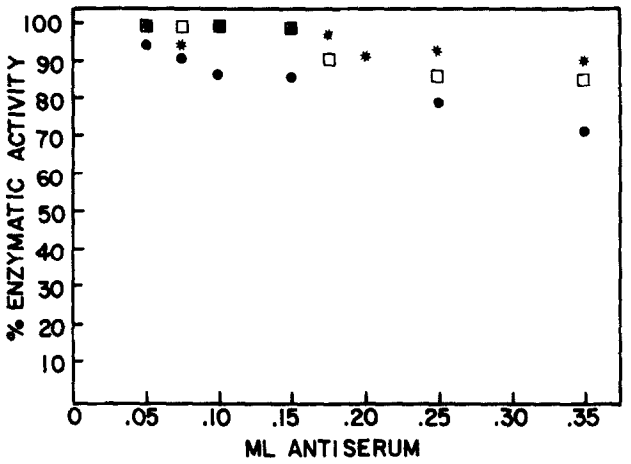

Figure 3: Enzymatic activity of C_1 and C_3 in the presence of increasing amounts of antiserum to C_1. Low concentration of enzyme and antiserum; assay carried out in the presence of precipitate.

• - 5βDHT as substrate, enzyme C_1
* - cyclohexanone as substrate, enzyme C_1
□ - cyclohexanone as substrate, enzyme C_3

Furthermore, the curves for decrease in activity were virtually identical for the steroid with enzyme C_1 and for cyclohexanone with enzymes C_1 and C_3.

When the enzymes were reacted with C_1 antiserum at a 1:10 dilution of both enzyme and antiserum, the results were qualitatively very similar to the experiments with concentrated solutions. Although the decrease in steroid activity was not so great in the dilute unspun mixture (Figure 3) as in the more concentrated one (Figure 1), the steroid reduction was still affected more than was cyclohexanone. When the precipitate was centrifuged (Figure 4) the activity curves for the steroid activity of C_1 and the cyclohexanone activity of C_1 and C_3 were virtually superimposable with each other and with the curve shown in Figure 2.

From these results it may be concluded that the C_1 antiserum primarily affects the catalytic activity of the steroid site of C_1 but not the cyclohexanone site of C_1 or of C_3. Loss of cyclo-

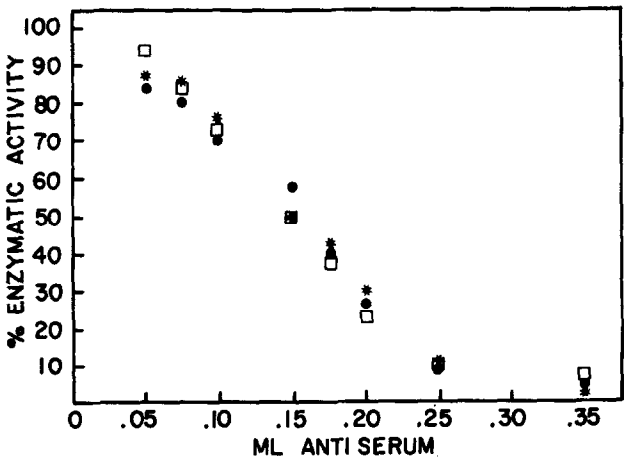

Figure 4: Enzymatic activity of C_1 and C_3 in the presence of increasing amounts of antiserum to C_1. Low concentration of enzyme and antiserum; precipitate removed.

● - 5βDHT as substrate, enzyme C_1
* - cyclohexanone as substrate, enzyme C_1
□ - cyclohexanone as substrate, enzyme C_3

hexanone activity occurs only when the precipitate is removed. This is in marked contrast to antiserum formed against C_3, which has its most pronounced effect against the cyclohexanone sites.

The identical precipitation curves for enzymes C_1 and C_3 with C_1 antiserum offers additional evidence for the structural similarity and close relationships of these two enzyme forms.

Procedure:

Antiserum Preparation - Preparation of antiserum was carried out as described in the previous paper, except that ADH (C_1) was stabilized in glycerin - .02M tris-HCl buffer (1:1) prior to dilution with Freund's adjuvant.

Interaction of Antiserum with ADH-C_1 and -C_3 - The procedure was precisely as detailed in the preceding communication, except that protein concentration was kept constant at 14 mg/7.25 ml by the addition of bovine serum albumin. In the reaction with concentrated enzyme and antiserum, the range of antiserum studied was 0-0.2 ml, while in the more dilute experiment it was 0-0.35 ml.

REFERENCES

Pietruszko, R. and Ringold, H. J., Biochem. Biophys. Res. Comm. (1968)