# LIGAND INDUCED MOLECULAR WEIGHT TRANSITIONS IN RHODOPSEUDOMONAS PALUSTRIS FRUCTOSE 1, 6-DIPHOSPHATASE

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SUMMARY. Fructose 1, 6-diphosphatase (EC 3.1.3.11) from the photosynthetic bacterium, Rhodopseudomonas palustris, undergoes reversible dissociation from a catalytically diminished 130,000 molecular weight species to a catalytically enhanced 65,000 molecular weight form in the presence of the allosteric ligands, fructose diphosphate and $Mn^{++}$ ions. In addition, alkaline pH was observed to mimic the effect of allosteric ligands on the quaternary structure of the enzyme. The mild conditions (25°C, pH 7.4) under which dissociation is induced by allosteric ligands suggest that these molecular weight transitions with the accompanying changes in catalytic activity may occur in vivo and play a role in regulating the Calvin-Bassham carbon reduction cycle.

Previous reports (1, 2) from this laboratory have demonstrated that fructose 1, 6-diphosphatase from the photosynthetic bacterium, Rhodopseudomonas palustris, is allosterically regulated by substrate (fructose 1, 6-diphosphate), cofactor ($Mn^{++}$ ions), and inhibitor (GTP). Although these ligands are structurally quite heterogenous and exert different effects on enzymatic activity, the cooperative interactions exhibited by all these modifiers at neutral pH are almost completely abolished at alkaline hydrogen ion concentration (1, 2). Ligand cooperativity was also observed (1, 2) to be greatly diminished in the presence of saturating concentrations of substrate or cofactor. We now report the results of

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gel filtration studies which demonstrate that R. palustris fructose diphosphatase undergoes reversible dissociation from a 130,000 molecular weight form to a catalytically enhanced 65,000 molecular weight species in the presence of alkaline pH, or in the presence of either substrate or cofactor at neutral hydrogen ion concentration.

## MATERIALS AND METHODS

All reagents were analytical grade and purchased from Sigma.

R. palustris fructose 1, 6-diphosphatase was purified as previously described (1, 2) and stored at -20°C in the presence of 0.1 mM $Mn^{++}$ ions. Only enzyme stored for less than 7 days was employed, owing to the irreversible formation of a 65,000 molecular weight species after storage for longer than 14 days (2).

Fructose diphosphatase activity was assayed in a standard reaction mixture containing in a volume of 1.0 ml: 100 mM Tris-HCl buffer pH 8.5, 0.5 mM fructose 1, 6-diphosphate, 0.1 mM $MnCl_2$, 1.0 mM dithiothreitol, 1.0 mM $NADP^+$, 20 μg phosphoglucoisomerase, and 4 μg glucose 6-phosphate dehydrogenase. Enzyme activity was monitored on a Gilford 24000 Spectrophotometer by following the reduction of $NADP^+$ at 340 nm. One enzyme unit is defined as the amount catalyzing the formation of 1 μmole of fructose 6-phosphate per minute. Specific activity is defined as enzyme units per mg of protein.

## RESULTS

### Effect of alkaline pH on fructose diphosphatase molecular weight

Results of molecular weight studies using Sephadex G-150 columns are presented in Figure 1. The gel filtrations were performed at neutral (pH 7.4) and alkaline (pH 8.5) hydrogen ion concentrations. All studies

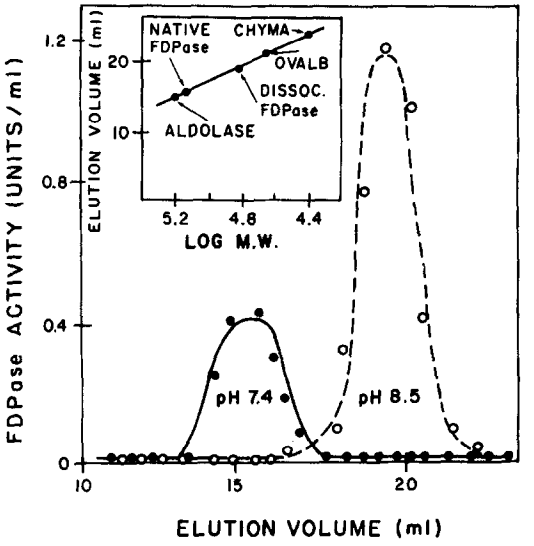


Figure 1. Effect of varying hydrogen ion concentration on fructose diphosphatase molecular weight. Fructose diphosphatase activity is plotted against the elution volume from a Sephadex G-150 column (0.9 cm x 55 cm). The columns were equilibrated with 0.1 M Tris-HCl buffer pH 7.4 or pH 8.5 containing 1 mM dithiothreitol for at least 24 hours before 0.3 mg of pure enzyme in 0.3 ml were layered on the top of each column. Before application to the Sephadex columns the enzyme samples were dialyzed against 0.1 M Tris-HCl pH 7.4 or pH 8.5 containing dithiothreitol and subsequently incubated at 25°C for 30 minutes. The temperature of all columns was maintained between 22°C and 25°C. Enzymatic activity was assayed employing the standard reaction mixture at pH 8.5. The inset presents the standard proteins employed for calibration of the columns. The abbreviations employed are: CHYMA, chymotrypsinogen A; OVALB, ovalbumin.

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were performed at an ambient temperature (22-25°C) to avoid possible effects of cold (2) on the molecular weight of the enzyme and to enhance the possibility of correlating the quaternary structure of the enzyme with the results of kinetic experiments (2) carried out at room temperature. As shown in Figure 1, alkaline pH dissociates the enzyme from the molecular weight of 130,000 (elution volume, Ve = 15.6 ml), which is characteristic at pH 7.4, to a 65,000 molecular weight species (Ve = 19.3 ml). The data also demonstrates that the dissociated form of the enzyme is approximately three times as active as the native 130,000 molecular

weight form. This is indicated by a specific activity of 8.8 for the former and 2.8 for the latter. The lack of ligand cooperative interactions at alkaline pH (2) is consistent with the explanation that the 65,000 molecular weight species induced by alkaline pH has fewer ligand binding sites than the 130,000 molecular weight form present under neutral conditions. It is of interest to note that the catalytic properties of rabbit liver fructose diphosphatase are also altered by shifts in hydrogen ion concentration (3, 4).

Effect of allosteric modifiers on fructose diphosphatase molecular weight

Results of kinetic experiments presented in previous reports from this laboratory (2) suggested saturating fructose 1, 6-diphosphate concentrations (0.5 mM) or $K_m$ levels of $Mn^{++}$ ions (0.02 mM) could promote dissociation of R. palustris fructose diphosphatase. To further study this concept, gel filtrations were performed at neutral pH in the presence of these modifiers. The results of these experiments are presented in Figure 2. The data clearly demonstrate that $Mn^{++}$ ions (Figure 2A, Ve = 15.4 ml) or fructose diphosphate (Figure 2B, Ve = 15.6 ml) promote dissociation of the enzyme to the 65,000 molecular weight species. Furthermore, the 130,000 molecular weight form is much less active (specific activity = 2.6-3.0) than the 65,000 molecular weight species (specific activity = 8.5-8.9). These molecular weight transitions correlate well with previous reports (2) which showed that while saturating fructose diphosphate concentrations were required to abolish $Mn^{++}$ cooperative interactions, only $K_m$ levels of $Mn^{++}$ ions were required to abolish substrate cooperativity. Studies (Figure 2B) performed at saturating concentrations of both cofactor and substrate showed that the molecular weight of R. palustris fructose diphosphatase re-

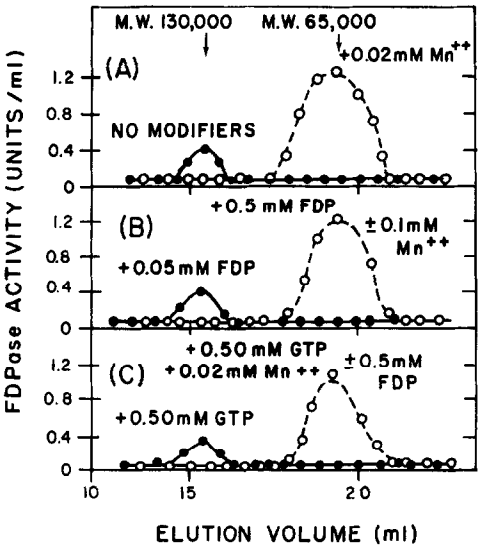


Figure 2. Effect of allosteric modifiers on fructose diphosphatase molecular weight. Fructose diphosphatase activity is plotted against the elution volume from a Sephadex G-150 column (0.9 cm x 55 cm). The columns were equilibrated with 0.1 M Tris-HCl buffer pH 7.4 containing 1 mM dithiothreitol with the appropriate modifier for at least 24 hours before 0.3 mg of pure enzyme in 0.3 ml were layered on the top of each column. Before application to the Sephadex columns the enzyme samples were incubated in 0.1 M Tris-HCl pH 7.4 containing 1 mM dithiothreitol and the appropriate modifier at 25°C for 30 minutes. The temperature of all columns was maintained between 22°C and 25°C. Enzymatic activity was assayed employing the standard reaction mixture at pH 8.5.

mained 65,000 under this condition. The allosteric inhibitor, GTP, does not cause the association or dissociation of the enzyme under a variety of experimental conditions (Figure 2C). Since GTP enhances substrate and cofactor cooperative interactions (2) this finding suggests GTP induces subtle changes in enzyme conformation not detectable employing gel filtration.

To ascertain if the observed dissociation was reversible, dissociated enzyme was dialyzed against 0.1 M Tris-HCl pH 7.4 containing 1 mM dithiothreitol and placed on a Sephadex G-150 column (0.9 cm x 55 cm) previously equilibrated with the dialysis buffer. Enzymatic activity was

recovered in an elution volume of 15.6 ml corresponding to that of the native enzyme, indicating the dissociation phenomenon is a reversible process. The method employed for determining the molecular weight is not sufficiently rapid to permit a kinetic analysis of reassociation.

The results presented here clearly indicate R. palustris fructose diphosphatase undergoes a reversible dissociation which is induced by saturating fructose diphosphate levels or $K_m$ concentrations of $Mn^{++}$ ions at neutral pH and 25°C. In the absence of allosteric modifiers, alkaline pH is also capable of inducing formation of the catalytically enhanced 65,000 molecular weight species. Substantial conformational changes induced by ligands have also been reported in fructose diphosphatases from rabbit liver (5, 6) and Candida utilis (7). The mild conditions (25°C, pH 7.4) under which dissociation of the R. palustris enzyme is induced by allosteric ligands suggest that these molecular weight transitions with the accompanying changes in catalytic activity may occur in vivo and play a role in regulating the Calvin-Bassham carbon reduction cycle.

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