Dissociation-Produced Loss of Regulatory Control of Homoserine Dehydrogenase of *Rhodospirillum rubrum*. II. Some Properties of the Regulatable and Nonregulatable Forms*

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ABSTRACT: Dissociation of homoserine dehydrogenase of *Rhodospirillum rubrum* produces a form insensitive to threonine inhibition but fully active. An analog of threonine, β-hydroxynorvaline, also inhibits the associated form of the enzyme and shares with threonine specificity for this form and reversal of its effect by isoleucine, methionine, and norleucine. Serine inhibits with distinctly different characteristics in that both associated and dissociated enzyme are affected and there is little or no reversal by the above liberators of threonine and β-hydroxynorvaline inhibition. We thus conclude that serine binding is isosteric rather than allosteric. The ability of the liberators of threonine inhibition to activate the reaction in the reverse direction (homoserine oxidation) is also lost upon dissociation of the enzyme. The characteristics of Hg^{2+} inactivation of associated and dissociated enzyme are different in rate, extent, and reversibility. Relatively minor differences in the substrate saturation curves were observed between the forms of the enzyme.

In the previous paper (Mankovitz and Segal, 1969b) we reported that homoserine dehydrogenase of *Rhodospirillum rubrum* undergoes a dissociation to a form which is insensitive to threonine inhibition but is fully active. In this paper we report the results of a comparison of a number of properties of the associated and dissociated forms of the enzyme.

Methods

The enzyme was prepared and assayed as described in the previous paper (Mankovitz and Segal, 1969b). As prepared (in buffer A, *i.e.*, high ionic strength buffer), the enzyme was nearly fully in the associated (threonine sensitive) state (σ = 0.84–0.87[1]). In some experiments a dilution of the stock enzyme solution of tenfold was desirable, which was carried out in 1.5 M KCl to maintain the enzyme in the associated state (σ = 0.90) (*cf.* Figure 6, Mankovitz and Segal, 1969b).

To obtain the dissociated form, the enzyme solution was passed through Sephadex G-25 in buffer B (low ionic strength buffer) and further diluted with buffer B to give fully desensitized enzyme. Enzyme preincubated in buffer B was assayed in buffer B to minimize resensitization during the assay incubation. The difference in buffer ionic strength had no effect on initial rates.

The enzyme concentrations during preincubation are indicated in the legends with each experiment.

Results

Threonine and β-Hydroxynorvaline Inhibition. Figure 1 presents curves of threonine and β-hydroxynorvaline inhibition with associated and dissociated enzyme. The insensitivity of dissociated enzyme to these inhibitors may be noted. β-Hydroxynorvaline is a considerably less effective inhibitor than threonine and its saturation curve shows no evidence of sigmoidicity. Nevertheless, it shares with threonine the property of specificity for the associated form of the enzyme and reversal of its inhibition by isoleucine, methionine, and norleucine (see below). A Hill plot of the threonine inhibition data for associated enzyme in Figure 1A gives a value of 2.4, close to the Hill coefficient for threonine *sensitization* (*cf.* Figure 3, Mankovitz and Segal, 1969b).

Serine Inhibition. The characteristics of serine inhibition were distinctly different from those of threonine and β-hydroxynorvaline in that the sensitivity of the enzyme to serine showed little or no dependence upon its state of aggregation (Figure 2). Figure 2 also indicates the lack of dependence of serine inhibition upon TPNH concentration and its reversal with increased aspartic β-semialdehyde concentration. The biphasicity of serine inhibition at the elevated aspartic β-semialdehyde concentration is reminiscent of a similar characteristic of threonine inhibition (Datta and Gest, 1965).

The finding that serine inhibition, like activity, is virtually invariant with respect to changes in the state of aggregation of the enzyme suggest that serine is an isosteric, rather than an allosteric, inhibitor. If this is the case, serine inhibition, in contrast to threonine (Sturani *et al.*, 1963; Datta and Gest, 1965) and β-hydroxynorvaline inhibition, should not be reversed by isoleucine, methionine, and norleucine. This prediction is borne out as demonstrated in Table I.

Isoleucine Activation of the Reverse Reaction. Sturani *et al.* (1963) and Datta and Gest (1965) have noted that isoleucine, methionine, and norleucine are activators of the reverse reaction (homoserine oxidation) as well as liberators of threonine inhibition of aspartic β-semialdehyde reduction. As shown

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[1] σ is a measure of threonine sensitivity, ranging from 0 for fully dissociated enzyme to 0.90 for fully associated enzyme (Mankovitz and Segal, 1969b).

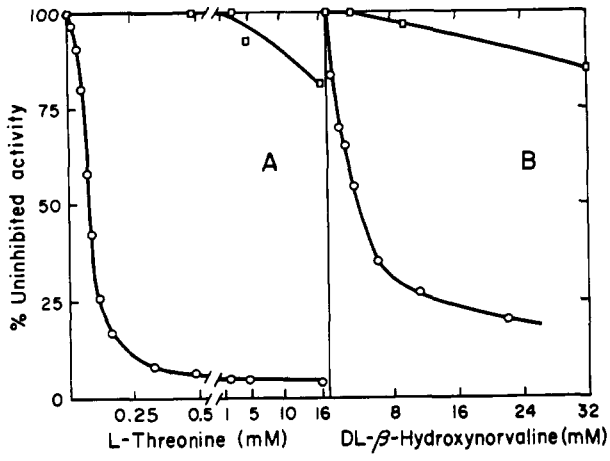

FIGURE 1: Effect of threonine and β-hydroxynorvaline on associated and dissociated homoserine dehydrogenase. A represents the threonine inhibition curves and B the β-hydroxynorvaline inhibition curves. Circles are for associated enzyme (1.5 units/ml) and squares for dissociated enzyme (0.14 unit/ml) (see Methods).

in Table II, the activating ability of isoleucine is specific for the associated form of the enzyme.

Substrate Saturation Curves. Figure 3 is a plot of homoserine dehydrogenase activity as a function of L-aspartic β-semialdehyde concentration (Figure 3A) and TPNH concentration (Figure 3B) for both associated and dissociated enzyme. The properties of the two forms of the enzyme in this regard were qualitatively very similar. However, there was a noticeably greater sensitivity of the dissociated form to inhibition by high aspartic β-semialdehyde levels.

Inactivation by Hg^{2+}. The characteristics of Hg^{2+} inactivation of the associated and dissociated forms of the enzyme were different in several distinct ways (Figure 4). The response of the former species was immediate, relatively small in mag-

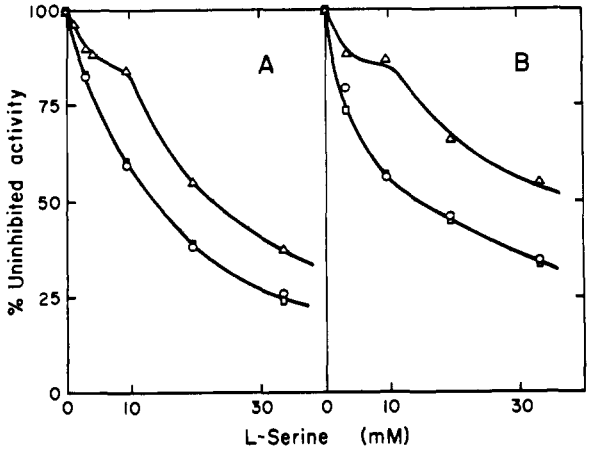

FIGURE 2: Effect of serine on associated and dissociated homoserine dehydrogenase. A demonstrates the inhibition of associated enzyme (1.4 units/ml) and B of dissociated enzyme (0.14). Circles represent assays at standard substrate concentrations (59 μM L-aspartic β-semialdehyde and 16 μM TPNH), squares are with elevated TPNH (48 μM), and triangles are with elevated aspartic β-semialdehyde (194 μM).

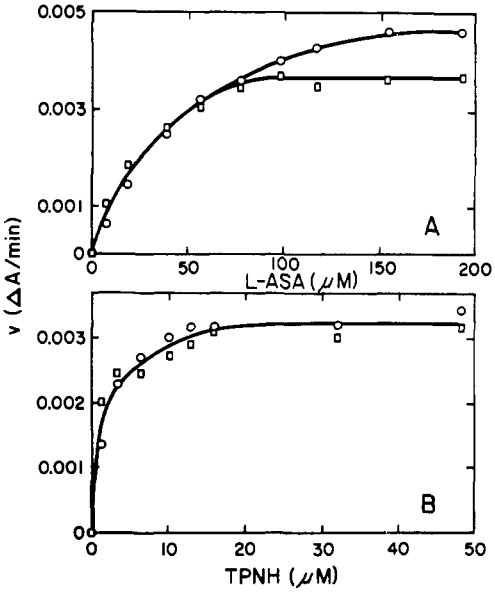

FIGURE 3: Dependence of velocity upon substrate concentration. Circles refer to the associated form of the enzyme (1.5 units/ml) and squares to the dissociated form (0.15 unit/ml). Equal amounts of the two forms of the enzyme were used (1 μl of associated enzyme or 10 μl of dissociated enzyme). In A the TPNH concentration was 16 μM and the L-aspartic β-semialdehyde concentration was varied as shown. In B the L-aspartic β-semialdehyde concentration was 58 μM and the TPNH concentration was varied as shown.

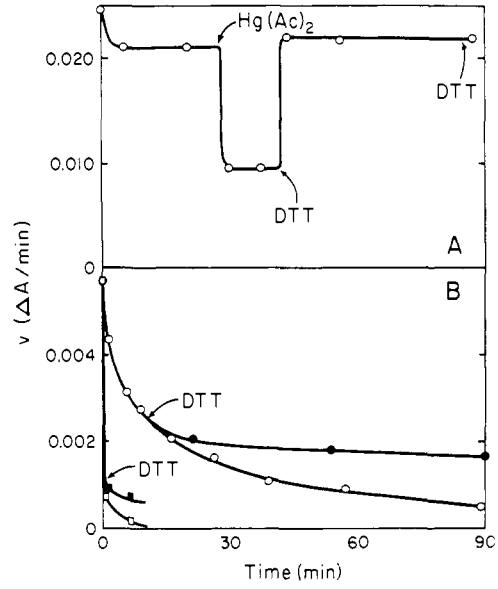

FIGURE 4: Inactivation of HDH by Hg^{2+}. (A) One micromole of mercuric acetate per milliliter of enzyme solution (associated form, 12.5 units/ml) was added at zero time and at the point marked "$HgAc_2$." Two micromoles of dithiothreitol per milliliter was added at each of the points marked "DTT;" 1-μl aliquots were taken for assay at the times indicated. (B) At zero time either 1 (circles) or 2 μmoles (squares) of mercuric acetate per ml of enzyme solution (dissociated form, 0.14 unit/ml) was added. At the points marked "DTT," 2 μmoles of dithiothreitol/ml was added to one set of tubes (filled symbols); 20-μl aliquots were taken for assay at the times indicated.

TABLE I: Lack of Reversal of Serine Inhibition of Homoserine Dehydrogenase by Liberators of Threonine and β-Hydroxynorvaline Inhibition.

	Activity (ΔA/min)			
	Inhibitor			
Liberator	None	9.7 mM L-Serine	0.081 mM L-Threonine	3.2 mM DL-β-Hydroxynorvaline
None	0.0028, 0.0027[a]	0.0017, 0.0015[a]	0.0017, 0.0027[a]	0.0015, 0.0027[a]
12 mM L-isoleucine	0.0027, 0.0026[a]	0.0019, 0.0015[a]	0.0027	0.0025
12 mM L-methionine	0.0025, 0.0025[a]	0.0019, 0.0014[a]	0.0023	0.0023
24 mM DL-norleucine	0.0026, 0.0026[a]	0.0019, 0.0014[a]	0.0027	0.0025

[a] These rates were with dissociated enzyme, all others were with the enzyme in the associated form (see Methods).

TABLE II: Activation by Isoleucine of Homoserine Oxidation.[a]

Enzyme Form	Activity (ΔA/min)		% Activation
	−Isoleucine	+Isoleucine	
Associated	0.0043	0.0098	128
Dissociated	0.0018	0.0020	11

[a] Assay mixture contained 30 mM Tris (pH 8.3), 0.1 M KCl, 1 mM EDTA, 4.8 mM L-homoserine, 125 μM TPN, 2.1 mM L-isoleucine (when added), and 5 μl of associated enzyme (11 units/ml) or 75 μl of dissociated enzyme (0.32 unit/ml).

nitude, and almost totally reversible by dithiothreitol, whereas with the latter form the inactivation reaction was slower, greater in extent, and slowed but not reversed by dithiothreitol.

Discussion

It appears clear from these results that all of the allosteric control properties of this enzyme disappear upon its dissociation, with little effect on the properties of the catalytic site. The former include the end-product inhibition by threonine (as well as β-hydroxynorvaline) and the liberation and/or activation by isoleucine and methionine (as well as norleucine). Since all of these ligands share the characteristics of ineffectiveness with dissociated enzyme and of shifting the equilibrium toward the associated state (Mankovitz and Segal, 1969b), it seems likely that their binding site is a common one. Among the "invariant" properties are activity *per se* and serine inhibition. Insufficient knowledge of the metabolic economy of the organism is available to speculate on the physiological significance of these findings.

That a dramatic conformational difference exists between the associated and dissociated forms of the enzyme can also be deduced from the differences in the characteristics of Hg^{2+} inactivation. There is no doubt that the opportunities for structural studies which the availability of homogeneous enzyme would provide could offer great insight into these interactions.

References

Datta, P., and Gest, H. (1965), *J. Biol. Chem. 240*, 3023.

Mankovitz, R., and Segal, H. L. (1969a), *FEBS Letters 3*, 44.

Mankovitz, R., and Segal, H. L. (1969b), *Biochemistry 8*, 3757.

Sturani, E., Datta, P., Hughes, M., and Gest, H. (1963), *Science 141*, 1053.