BASIS FOR AMP ACTIVATION OF "BIODEGRADATIVE" THREONINE DEHYDRASE FROM ESCHERICHIA COLI[1]

A. T. Phillips[2] and W. A. Wood

Department of Biochemistry, Michigan State University, East Lansing, Michigan

Received March 30, 1964

The "biodegradative" threonine dehydrase of anaerobically grown Escherichia coli has long been characterized by a marked AMP activation, and by a lack of isoleucine inhibition (Wood and Gunsalus, 1949; Umbarger and Brown, 1957). Its formation requires the presence of an inducer, threonine or serine, and exclusion of glucose and oxygen (Umbarger, 1961). Large amounts are induced relative to the amount of "biosynthetic" threonine dehydrase (required for isoleucine formation) which is found in this organism. For this reason, the biodegradative dehydrase is considered to play an important role in anaerobic energy production by supplying a substrate for α-keto acid oxidation (Tokushige et al., 1963; Walker, 1958) and for regeneration of NAD^+ by α-keto acid reduction (Umbarger, 1961). In E. coli, regulation of these reactions is possible by the control which AMP exerts on threonine dehydrase. Recently, Monod et al. (1963) have reviewed examples of metabolic control brought about by "allosteric" effectors; in a number of cases adenine nucleotides have functioned in this capacity.

Studies with T_2O, D_2O and specifically tritiated threonine have indicated that dehydration proceeds as expected for α,β-elimination

[1] Supported in part by grants from the United States Atomic Energy Commission and the National Institutes of Health. Contribution No. 3338 of the Michigan Agricultural Experiment Station.

[2] Predoctoral Fellow, National Institutes of Health.

reactions involving pyridoxal phosphate (Phillips and Wood, manuscript in preparation). No suggestion for AMP involvement in the reaction mechanism per se has been obtained. Experiments to be described here indicate a role of AMP in controlling enzyme activity by altering the structure of the dehydrase.

Experimental

Threonine dehydrase was obtained from E. coli, Crookes strain, grown essentially as described by Wood and Gunsalus (1949). The method of purification will be detailed in a subsequent publication; for the present study, the enzyme was purified 50-fold over crude extracts and had a specific activity of 1,900 units per mg protein (Fig. 2, protein by the method of Lowry et al. (1951)).

Specificity of activation. Table I shows the relative effectiveness of a number of nucleotides and derivatives in activating threonine dehydrase. Each compound tested was adjusted to pH 8.0 prior to its addition to the assay mixture.

TABLE I

Specificity of Activation of Threonine Dehydrase

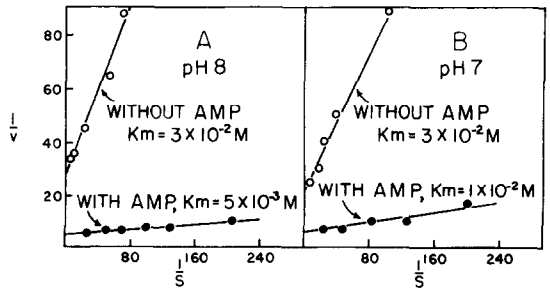
Compound Tested	% of Activity with AMP
AMP	100
CMP	48
dAMP	38
2'-AMP or 3'-AMP	27
ADP or ATP	20
GMP	17
UMP or IMP	4
3', 5'-cyclic AMP	0
Adenosine monosulfate	0
Adenosine monoacetate	0
Polyadenylic acid (2 mg/ml)	0

Each assay in a microcuvette contained the nucleotide to be tested, 1 μmole of glutathione, 0.08 μmole of NADH, 0.025 mg of muscle lactic dehydrogenase (Worthington), 15 μmoles of potassium phosphate buffer, pH 8.0, and enzyme. After a 5 minute preincubation, 4 μmoles of L-threonine, pH 8, were added to initiate the reaction. The final volume was 0.20 ml. Absorbancy changes were measured at 340 mμ. The final concentration of activator was 5×10^{-3} M except where noted.

Effect of AMP on catalysis. The K_m and V_{max} for the reaction in the presence and absence of AMP was determined at pH 8.0, the reaction optimum (Figure 1a) and at pH 7.0, the optimum for enzyme stability (Figure 1b). At pH 8.0, K_m and V_{max} in the presence of AMP were 5×10^{-3} M and 0.27 ΔA per minute; in the absence of AMP, K_m and V_{max} were 3×10^{-2} M and 0.034 ΔA per minute. K_m was similar at pH 7.0 and 8.0 in the absence of AMP.

FIGURE 1

K_m and V_{max} Determinations at pH 7 and 8



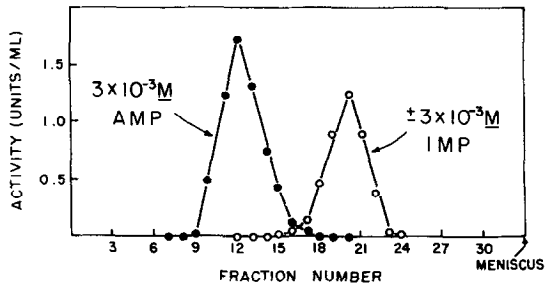
The AMP concentration was 3×10^{-3} M. Threonine concentrations are expressed in moles/liter. Velocities are expressed as Δabsorbancy/minute as determined in the NADH-lactic dehydrogenase-coupled assay. Data have not been corrected to account for differences in velocity at the different pH values.

Sucrose gradient centrifugation. Density gradient centrifugations, performed essentially as described by Martin and Ames (1961), were carried out for 17 hours at 35,000 rpm and 15°C. Well-characterized markers such as yeast alcohol dehydrogenase, horse liver alcohol dehydrogenase, aldolase, and 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase with known S_{20} values were placed on each gradient. Calculations of S_{20} were based on at least three markers and were in reasonably good agreement (± 0.3 S). Figure 2 shows the result of a typical run made in the presence of 3×10^{-3} M AMP or 3×10^{-3} M IMP. When no nucleotide was added to the gradient and care was taken to remove traces of AMP from

the enzyme preparation, the dehydrase migrated as in an IMP gradient. The S_{20} values obtained from these experiments were 7.6 S in 3×10^{-3} M AMP and 4.8 S in 3×10^{-3} M IMP. Assuming a spherical protein, these S_{20} values correspond to approximate molecular weights of 155,000 and 78,000, respectively (Martin and Ames, 1961).

FIGURE 2

Sucrose Gradient Centrifugation of Threonine Dehydrase



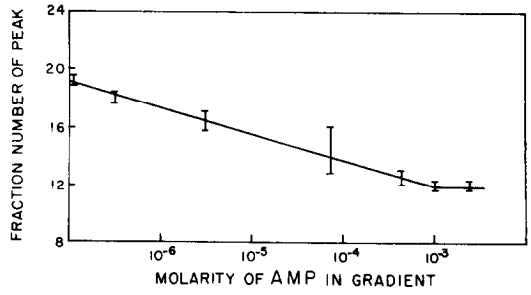
Marker peak positions were: yeast alcohol dehydrogenase, S_{20} = 7.5 S, fraction 13; horse liver alcohol dehydrogenase, S_{20} = 5.1 S, fraction 18; KDPG aldolase, S_{20} = 4.5 S, fraction 20. One unit of threonine dehydrase dehydrates 0.032 μmole of threonine per minute. The gradients were linear from 5 to 20% sucrose and contained nucleotide throughout. One-tenth ml sample was layered on each gradient. S_{20} values for yeast and liver alcohol dehydrogenase are taken from Sund and Theorell (1963); the value for KDPG aldolase was that reported by Meloche and Wood (1963).

Since partial activation was noted with CMP, a gradient containing this nucleotide (3×10^{-3} M) was prepared. This gradient showed threonine dehydrase to have an S_{20} of 7.5 S.

Figure 3 shows the relationship between the sedimentation velocity and AMP concentration at a fixed enzyme concentration. The absence of multiple peaks and the intermediate S_{20} values indicate that the two species are rapidly interconverted in the presence of limiting AMP. This is in agreement with the theory for sedimentation of reversibly interacting substances as discussed by Gilbert (1959). A pronounced broadening of the peaks is noted at intermediate concentrations of AMP.

FIGURE 3

Sedimentation Rate of Threonine Dehydrase as a Function of AMP Concentration



Conditions were the same as in Figure 2 except that the concentration of AMP was varied as indicated.

Discussion

The foregoing experiments indicate that AMP plays an important role in the enzymatic dehydration of threonine by controlling the affinity of the enzyme for substrate and thereby producing a more effective catalyst. We propose that this phenomenon is accomplished by the formation of a more active dimer since dimerization is observed under conditions similar to those prevailing in an enzymatic assay (i.e. pH 7-8, protein concentration 1-10 μg/ml, AMP concentration 3×10^{-3} M).

The reversible association in the presence of AMP, the specificity of activation and the marked changes in catalytic properties of threonine dehydrase upon AMP binding are all taken as evidence that AMP functions as a true allosteric effector in controlling the rate of threonine dehydration in anaerobically grown E. coli. The recent reports of Whiteley et al. (1964) and Hayaishi et al. (1963) of an ADP-activated threonine dehydrase from Clostridium tetanomorphum indicate that control of threonine catabolism and energy production by the levels of certain adenine nucleotides may be a widespread regulatory mechanism in anaerobiosis.

Bibliography

Gilbert, G.A., Proc. Roy. Soc. (A) 250, 377 (1959).
Hayaishi, O., Gefter, M., and Weissbach, H., J. Biol. Chem. 238, 2040 (1963).
Lowry, O.H., Roseborough, N.J., Farr, A.L., and Randall, R.J., J. Biol. Chem. 193, 265 (1951).
Martin, R.G., and Ames, B.N., J. Biol. Chem. 236, 1372 (1961).
Tokushige, M., Whiteley, H.R., and Hayaishi, O., Biochem. Biophys. Res. Comm. 13, 380 (1963).
Meloche, H.P., and Wood, W.A., Fed. Proc. 22, 411 (1963).
Monod, J., Changeux, J.P., and Jacob, F., J. Mol. Biol. 6, 306 (1963).
Sund, H. and Theorell, H., in The Enzymes, Vol. 7, Boyer, Lardy, and Myrback, Ed., Academic Press, 1963.
Umbarger, H.E., and Brown, B., J. Bacteriol. 73, 105 (1957).
Umbarger, H.E., Cold Spr. Harb. Symp. Quant. Biol. 26, 301 (1961).
Walker, D.J., Biochem. J. 69, 524 (1958).
Whiteley, H.R., and Hayaishi, O., Biochem. Biophys. Res. Comm. 14, 143 (1964).
Wood, W.A., and Gunsalus, I.C., J. Biol. Chem. 181, 171 (1949).