COENZYME DISSOCIATION, A POSSIBLE DETERMINANT OF SHORT HALF-LIFE OF INDUCIBLE ENZYMES IN MAMMALIAN LIVER*

Gerald Litwack and Sheila Rosenfield

Fels Research Institute and Department of
Biochemistry
Temple University School of Medicine
Philadelphia, Pa. 19140

Received March 19, 1973

SUMMARY

Corticosteroid inducible, rapidly turning-over ($t_{\frac{1}{2}}$ = 12 min to 3 hrs) enzymes of rat liver cytosol are complex enzymes with dissociable coenzymes. Enzymatic activity can be used to measure the relative rate of coenzyme dissociation. A comparison of rapidly inducible compared to relatively uninducible complex enzymes shows that the relative rate of coenzyme dissociation aligns with the shortness of the $t_{\frac{1}{2}}$ of the enzyme suggesting that coenzyme dissociation may be a limiting step in the degradation process of these enzymes.

The specificity of the anabolic actions of corticosteroids in the liver cell involves the enhanced protein synthesis of a relatively small group of cytoplasmic enzymes whose turnover is rapid. So far, all of the enzymes within this group which have very short half-lives (i.e. 12 minutes to 3 hrs) are complex enzymes containing dissociable coenzymes (1). When steroid inducible enzymes from liver cytoplasm are compared with similar non-inducible or poorly inducible complex enzymes of similar molecular weight sometimes having a like catalytic reaction mechanism as well as in some cases, the same coenzyme and existing in the same subcellular compartment, it is shown that the non-inducible enzymes have long half-lives and non-dissociating coenzymes (1). In this paper we review these properties of both inducible and non-inducible enzymes and show that, for the enzymes examined thus far, only those with dis-

*Supported by research grants AM-08350 and AM-13535 from the National Institute of Arthritis, Metabolic and Digestive Diseases and by CA-12227 to the Fels Research Institute.

sociable coenzymes are rapidly inducible and the relative rate of dissociation of co enzyme appears to align with the shortness of the half-life of the enzyme. Therefore, dissociation of coenzyme may be the rate-limiting step conditioning the apoenzyme for degradation (proteolysis). Katanuma et al.(2) have discovered an enzyme which appears to inactivate certain pyridoxal-P activated enzymes in their apo-form, such as apo-ornithine decarboxylase. Although this enzyme is located in skeletal muscle and in small intestine, but not in liver, the general conclusion that inactivation involves primarily the apo-enzyme following co-enzyme dissociation aligns with the hypothesis advanced here. Inevitably, the enzyme degradation process in which coenzyme dissociation can be considered as the first step, may control the rate of enzyme synthesis, at least under unperturbed conditions. In confirmation of this idea, certain nonhormonal inducers stimulate enzyme activity by preventing coenzyme dissociation (3). A correlation between rapidly turning-over enzymes and their susceptibility to the action of endopeptidases has been established in liver (4) as well as in bacteria (5). One suggestion is that protein conformations may determine the half-life of all proteins in vivo (5). This is in keeping with the data and conclusions drawn in the present study which infer that the apoenzyme would have a different conformation than the holoenzyme. The relationship between coenzyme dissociation and susceptibility to proteolytic attack seems especially appropriate since cofactors have been shown to protect enzymes from proteolytic digestion (4-7).

The specific events involved in enzymatic degradation following dissociation of coenzyme are as yet unexplained. It is not unlikely that degradation proceeds through conversion into enzyme subunits (8) and the molecular size of these subunits may form the selective basis for further degradation (9-11). We assume that the rates of conversion to subunits and their subsequent breakdown are slower than the rate of coenzyme dissociation.

Properties of inducible enzymes of liver cytosol. In Table 1 are compared the properties of inducible and similar but relatively non-inducible enzymes of rat liver cytosol. There is a correlation between coenzyme disso-

Table 1 Comparison of inducible versus relatively noninducible complex enzymes of liver cytosol

Enzyme	Mol. Wt.	Mol. Wt. of subunit	Rapidly inducible by corticosteroid	Half-life (days)	Cofactor	Dissociability of cofactor
Ornithine decarboxylase (E.C. 4.1.1.17)	100,000(12)	undetermined	+	0.01 (13)	pyridoxal-P	+++ (this paper)
Tyrosine aminotransferase (E.C. 2.6.1.5)	~100,000(14) to 115,000	25,000(4) to 32,000(14)	+	0.08 (15)	pyridoxal-P	++ (this paper and 16,17)
Tryptophan oxygenase (E.C. 1.13.1.12)	122,000(18)	31,000(18)	+	0.10 (19)	hematin	++ (20-22)
Alanine aminotransferase (E.C. 2.6.1.2)	114,000(23)	undetermined*	-	3-3.5(25,26)	pyridoxal-P	- (1)
Aspartate aminotransferase (E.C. 2.6.1.1)	110,000(27)**	55,000***	-	11 (29)	pyridoxal-P	- (this paper, 16,30)

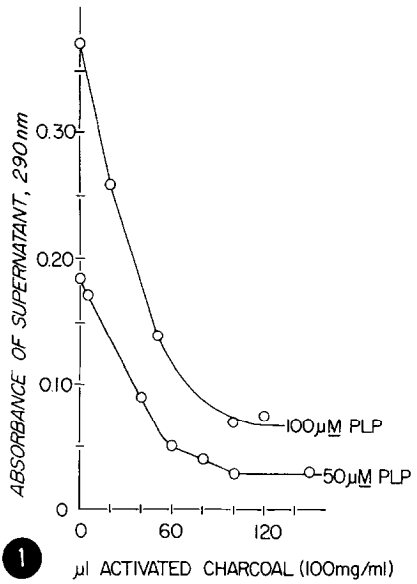
*There are 2 moles pyridoxal-P/mole of enzyme (24), therefore subunit mol. wt. may be 57,000.

**Data are for beef liver.

***Probable from data on heart enzymes (28).

ciability, short half-life and inducibility by corticosteroids. This could be explained on the basis that for complex enzymes coenzyme dissociation is the rate-limiting step in enzyme degradation. Consequently, a greater rate of dissociation of coenzyme would determine a shorter half-life of the enzyme. Since the usual approaches to the measurement of the rate of coenzyme dissociation, such as by equilibrium dialysis were unsuccessful in the case of tyrosine aminotransferase (31), a new method was developed for this measurement.

Relative rates of coenzyme dissociation. The degree of functionally bound coenzyme can be measured by assaying for enzymatic activity in the absence and presence of added coenzyme (16). The extent of coenzyme dissociation could be determined in the same way providing endogenous coenzyme, in this case pyridoxal-P, which was not bound to the enzyme could be quickly removed from solution. This was achieved by the use of activated charcoal (32) which was effective in removing nearly all of free pyridoxal-P from solution (Fig. 1). Of interest in this connection is the recent work of O'Leary and Malik who showed that binding of pyridoxal-P to bacterial apo-glutamate decarboxylase could be measured as well by enzymatic activity as by absorption spectroscopy, fluorescence or circular dichroism (33). Cytosols were prepared by previous methods from perfused livers of adrenalectomized male rats (110-200 g) obtained from Charles River Breeding Labs. (Boston) 4 hours after intraperitoneal injection of hydrocortisone (6 mg/100 g body weight) (34). Fifteen to 20 ml cytosol were used for each incubation at 37^{0}. If activated charcoal (32) was used to remove free pyridoxal-P from the cytosol, 125 µl charcoal suspension was added per ml of cytosol. When charcoal was used before incubation it was also added to aliquots of cytosol removed at indicated times of incubation to adsorb pyridoxal-P released from the bound state during the incubation. Charcoal suspension was added at the same level (125 µl/ml cytosol). Aliquots were 0.8 ml for the ornithine decarboxylase assay and 0.4 ml for assay of tyrosine aminotransferase and aspartate aminotransferase.



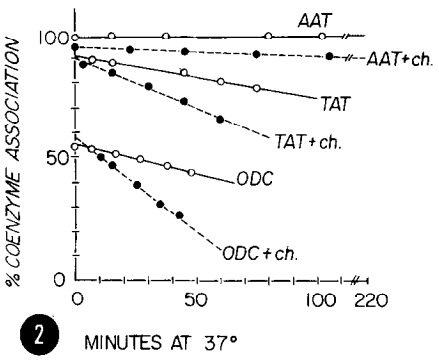


Fig. 1. Titration of free pyridoxal-P with activated charcoal. See text for details. PLP = pyridoxal-P.

Fig. 2. Relative rates of dissociation of coenzyme for aspartate aminotransferase (Asp. AT), tyrosine aminotransferase (TAT) and ornithine decarboxylase (OCD) in the presence or absence of activated charcoal (ch.). See text for details. In some cases it is useful to present data as % coenzyme association rather than as % coenzyme dissociation. % coenzyme association is 100 - (% dissociation) as defined in footnote to Table 2. Curves with open circles refer to systems in which charcoal was not used. Closed circles refer to systems in which the enzyme preparation was treated with charcoal to remove endogenous free pyridoxal-P before starting incubation. Subsequently, each aliquot taken at the indicated time was treated with charcoal again to remove free coenzyme dissociated during the course of incubation. The aliquot was then assayed for enzymatic activity with or without pyridoxal-P in the enzyme assay system (see text).

In incubations where charcoal was not used initially, it was also not added to portions taken at various times of incubation. When charcoal was added to the whole cytosol or portions taken at various times after incubation, the mixture was agitated on a Vortex mixer for 20 seconds and centrifuged at 600 x g for 2 min. The supernatant was recentrifuged at 12,000 x g for 5 min. and then filtered through glass wool and portions were assayed for protein content (absorbancy at 280 nm) and enzymatic activity in the presence and absence of pyridoxal-P. Ornithine decarboxylase activity was determined

Table 2 Comparison of the relative rates of coenzyme dissociation of corticosteroid inducible enzymes with their half-lives.

Enzyme	Relative coenzyme dissociation rate* (% coenzyme association/min)$^{-1}$	$t_{1/2}$ (hours)
aspartate aminotransferase	20	264
tyrosine aminotransferase	2.7	2.0
ornithine aminotransferase	1.4	0.25

*Determinations were made with and without charcoal treatment before incubation was started with each of the above enzyme assays. When each sample was taken from an incubation mixture originally treated with charcoal, charcoal was again added to the aliquot in order to adsorb pyridoxal-P further released during that incubation period. In systems where charcoal was not added to cytosol at the start of incubation, the aliquots taken at various times of incubation were likewise not treated with charcoal. Percent dissociation of coenzyme is defined as

$$100 - \left[\frac{\text{activity without coenzyme}}{\text{activity with coenzyme}} \times 100\right]$$

by a modified method of Russell and Snyder (35). 0.1 ml Cytosol was preincubated in the assay system without ^{14}C-ornithine for 10 min. 100,000 cpm ^{14}C-L-ornithine (carboxyl labeled specific radioactivity, 11.9 mCi/mmole) was added and incubated for 30 min. at 37°. 0.25 ml Hyamine hydroxide (1 M in methanol) was added to a well in the incubation vial and 1.0 ml 2 M citric acid was added to the assay medium. Incubation was continued for 30 min. at 37° to trap all of the $^{14}CO_2$ and the well was placed in a scintillation vial containing 10 ml Omnifluor in toluene (4 mg/ml) plus methanol (5:1, v/v). Tyrosine aminotransferase was determined as described previously using the Briggs method (31,36). The assay system in the absence of tyrosine was preincubated at 37° for 5 min. Tyrosine or buffer was then added, and incubation of the complete system was for 10 min. Aspartate aminotransferase was assayed according to established methods (37,38). The same procedure was used with respect to incubation and charcoal treatment for all of the enzyme

assays (see footnote, Table 2). The data for coenzyme dissociation for the 3 enzymes are shown in Figure 2 and the values for the relative rates of coenzyme dissociation (slopes of curves) using this method compared to the known half-life of the enzyme are presented in Table 2. The reciprocals of the relative rates of coenzyme association from the slopes of plots in Figure 2 align with the half-lives of the enzymes compared. As might be anticipated the alignment is not perfectly linear since all of the events in enzyme degradation following dissociation of coenzyme may occur at rates or under conditions which vary from one enzyme to the other, however, the generalization, at least for the examples cited, is supported. We suggest therefore, that coenzyme dissociation may be an important area to investigate as a starting point for unravelling the process of degradation of complex enzymes.

REFERENCES

1. Litwack, G. and Singer, S., (edited by Litwack, G.), Biochemical Actions of Hormones, Academic Press, New York, 2, 113 (1972).
2. Katanuma, N., Kominami, E., and Kominami, S., Biochem. Biophys. Res. Commun., 45, 70 (1971).
3. Singer, S. and Mason, M., Biochim. Biophys. Acta, 146, 452 (1967).
4. Bond, J.S., Biochem. Biophys. Res. Commun., 43, 333 (1971).
5. Goldberg, A.L., Proc. Nat'l. Acad. Sci. (U.S.A.), 69, 2640 (1972).
6. Grisolia, S., Physiol. Rev., 44, 657 (1964).
7. Schimke, R.A., Sweeney, E.W. and Berlin, C.M.,J.Biol. Chem., 240, 4609 (1965)
8. Litwack, G., Lichtash, E. and Diamondstone, T.I., Nature-New Biology, 237, 149 (1972).
9. Dehlinger, P.J., and Schimke, R.T., Biochem. Biophys. Res. Commun., 40, 1473 (1971).
10. Dehlinger, P.J. and Schimke, R.T., J. Biol. Chem., 246, 2574 (1971).
11. Glass, R.D. and Doyle, D., J. Biol. Chem., 247, 5234 (1972).
12. Ono, M., Inoue, H., Suzuki, F. and Takeda, Y., Biochim. Biophys. Acta, 284, 285 (1972).
13. Russell, D.H. and Snyder, S.H., Mol. Pharmacol., 5, 253 (1969).
14. Auricchio, F., Valeriote, F., Tomkins, G. and Riley, W.D., Biochim. Biophys. Acta, 221, 308 (1970).
15. Kenney, F.T., in Mammalian Protein Metabolism (edit. Munro, H.F.), Academic Press, New York, 4, 131 (1970).
16. Litwack, G. and Cleland, W.W., Biochemistry, 7, 2072 (1968).
17. Rosenberg, J.S. and Litwack, G., Physiol. Chem. and Phys., 3, 129 (1971).
18. Poillon, W.N., Maeno, H., Koike, K. and Feigelson, P., J. Biol. Chem., 244, 3447 (1969).
19. Schimke, R.T. and Doyle, D., Ann. Rev. Biochem., 30, 929 (1970).
20. Knox, W.E. and Piras, M.M., J. Biol. Chem., 242, 2959 (1967).
21. Piras, M.M. and Knox, W.E., J. Biol. Chem., 242, 2952 (1967).
22. Li, J.B. and Knox, W.E., J. Biol. Chem., 247, 7546 (1972).

23. Gatehouse, P.W., Hopper, S., Schatz, L. and Segal, H.L., J. Biol. Chem., 242, 2319 (1967).
24. Segal, H.L., and Matsuzawa, T., in Tabor, H. and Tabor, C.W., (editors), Methods in Enzymology, Academic Press, New York, 18, 153 (1970).
25. Segal, H.L. and Kim, Y.S., Proc. Nat'l. Acad. Sci., 50, 912 (1963).
26. Kim, Y.S., Molec. Pharmacol., 5, 105 (1969).
27. Morino, Y., and Wada, H., in Snell, E.E., Fasella, P.M., Braunstein, A.E., and Rossi Fanelli, A., (editors), Chemical and Biological Aspects of Pyridoxal Catalysis, Pergamon Press, New York (1963) p. 175.
28. Bertland, L.H. and Kaplan, N.O., Biochemistry, 7, 134 (1968).
29. Ogawa, Y., Kometani, Y. and Baba, Y., Chem. and Pharm. Bull. (Tokyo), 16, 1948 (1968).
30. Miller, J.E. and Litwack, G., J. Biol. Chem., 246, 3234 (1971).
31. Rosenberg, J.S. and Litwack, G., J. Biol. Chem., 245, 5677 (1970).
32. Baxter, J.D. and Tomkins, G.M., Proc. Nat'l. Acad. Sci., 68, 932 (1971).
33. O'Leary, M.H. and Malik, J.M., J. Biol. Chem., 247, 7097 (1972).
34. Singer, S. and Litwack, G., Endocrinology, 88, 1448 (1971).
35. Russell, D.H. and Snyder, S.H., Fed. Proc., 27, 642 (1968).
36. Canellakis, Z.N. and Cohen, P.P., J. Biol. Chem., 222, 53 (1956).
37. Amador, E. and Wacker, W.E.C., Clin. Chem., 8, 343 (1962).
38. Karmen, A., J. Clin. Invest., 34, 131 (1955).