BBA 63416

Factors affecting urea inactivation of NADP-specific β-isocitrate dehydrogenase

Several electrophoretically distinct forms of NADP-specific isocitrate dehydrogenase (*threo*-D_S-isocitrate:$NADP^+$ oxidoreductase (decarboxylating), EC 1.1.1.42) have been reported to occur in *Escherichia coli* grown under different culture conditions[1]. Since *E. coli* as well as most other bacterial species[2] contain only the NADP-specific form of this enzyme, it has been suggested[3] that the regulation of isocitrate metabolism may be controlled by multiple forms (isoenzymes) of this key enzyme under different growth conditions.

The α- and β-forms of NADP-specific isocitrate dehydrogenase, which occur in glucose-grown *E. coli*, have been separated and shown to exhibit marked differences with respect to urea inactivation[3]. A similar differential susceptibility to urea has been observed with the LDH-1 and LDH-5 isoenzymes of lactate dehydrogenase (L-lactate:NAD^+ oxidoreductase, EC 1.1.1.27)[4–6]. Although the phenomenon is not understood, the observation has been the basis of methods for determining lactate dehydrogenase patterns in serum[7,8].

The present studies provide an insight into the inactivation of the β-form of NADP-specific isocitrate dehydrogenase in 2.0 M urea.

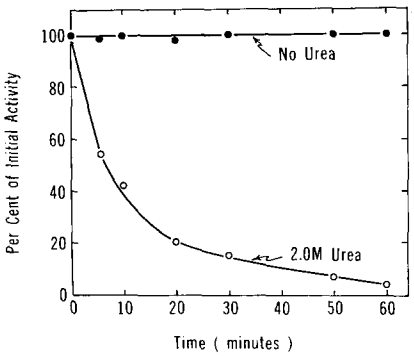
E. coli was grown aerobically in a simple mineral-salts medium containing 0.2% glucose as carbon source and cell-free extracts prepared as previously described[9]. The β-isocitrate dehydrogenase was isolated employing DEAE- and CM-Sephadex column chromatography as reported previously[3]. For enzyme assays, the change in absorbance at 340 mμ was followed using a Cary 14 spectrophotometer.

Protein concentrations were determined by measuring the absorbance at 215 and 225 mμ in a Beckman DU spectrophotometer according to the method described by MURPHY AND KIES[10].

0.5 ml of 4.0 M urea freshly prepared in 0.01 M phosphate buffer (pH 7.5) was added to 0.5 ml of the same buffer containing β-isocitrate dehydrogenase and the solution placed in an ice bath at 0°. Immediately after mixing, and at intervals thereafter, aliquots were assayed for enzymatic activity. The results are shown in Fig. 1 and are expressed as percent of activity in the control remaining at the times indicated. Under these conditions, 60% of the initial enzyme activity was lost after 10 min and only 5% remained after 1 h.

The effectiveness of NADP and dithiothreitol in protecting β-isocitrate dehydrogenase against inactivation in 2.0 M urea is shown in Fig. 2. A solution of the enzyme in 0.01 M phosphate buffer (pH 7.5) containing varying amounts of either NADP, dithiothreitol or a mixture of these was incubated in an ice bath at 0° for 30 min. An equal volume of 4.0 M urea was then added to each tube and incubation continued for 1 h at 0°. Aliquots were then removed and assayed for enzymatic activity. Appropriate controls containing no urea were also included.

Both dithiothreitol and NADP alone afford partial protection to the enzyme against inactivation in 2.0 M urea. In the presence of either 3.27 mM NADP or 105 mM dithiothreitol, the enzyme retained 45–50% of the initial activity after 1 h at 0° in 2.0 M urea. In the absence of either NADP or dithiothreitol, greater than



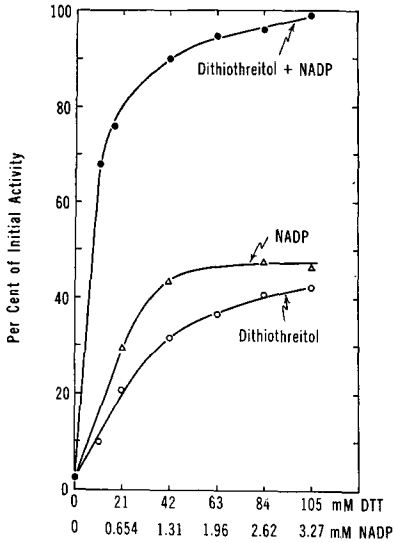


Fig. 1. Inactivation of β-isocitrate dehydrogenase at 0° in 2 M urea. The assay mixture contained (in μmoles): potassium phosphate buffer (pH 7.5), 150; $MnCl_2$, 0.5; NADP, 0.5, and DL-isocitrate (potassium salt), 2.5. The protein concentration was 6.0 μg and the total volume was 1.0 ml.

Fig. 2. Protection of β-isocitrate dehydrogenase against inactivation in 2 M urea by NADP and dithiothreitol (DTT). Assay conditions the same as described in Fig. 1.

95% of the initial catalytic activity was lost under the same conditions. At lower concentrations, the protective effect afforded by either NADP or dithiothreitol decreased.

In the presence of both NADP and dithiothreitol, the enzyme could be completely protected against inactivation in 2.0 M urea. As shown in Fig. 2, preincubation of β-isocitrate dehydrogenase with both NADP and dithiothreitol at a concentration of each which alone afforded 40–45% protection, resulted in complete protection of the enzyme. At lower concentrations also, a mixture of both NADP and dithiothreitol was found to be more effective than either compound alone in affording protection.

β-Isocitrate dehydrogenase in the presence of 2.0 M urea for 1 h loses greater than 90% of its catalytic activity. This is an irreversible inactivation in that the subsequent removal of urea by dialysis or by dilution does not restore the enzymatic activity.

Preincubation of β-isocitrate dehydrogenase with NADP, the specific cofactor required for catalytic activity, protects the enzyme from inactivation during subsequent exposure to urea. The maximum protection afforded by NADP, however, is 40–45% and further increasing the concentration of NADP does not result in any greater degree of protection. This observation suggests that one of the sites on the native protein which is affected by urea is involved with NADP binding.

As in the case of NADP, preincubation of β-isocitrate dehydrogenase with dithiothreitol resulted in a maximum of 40–45% protection against subsequent urea inactivation and higher concentrations of dithiothreitol did not result in any greater

degree of protection. This data suggests that in the absence of reducing conditions urea causes a partial disruption of the tertiary structure of the enzyme resulting in the exposure and subsequent oxidation of essential -SH groups. When this occurs in a reducing environment, the oxidation of these -SH groups is prevented and the enzyme maintains 40–45% of the native catalytic activity. If, however, the enzyme is either preincubated with NADP or if the exposure to urea is conducted under reducing conditions, the enzyme is only partially inactivated. Finally, if the native enzyme is preincubated with NADP and subsequently treated with 2.0 M urea under reducing conditions, there is no loss of catalytic activity.

This work was supported by grants from the National Science Foundation (GB-7396) and National Institutes of Health (5 RO1A1 03866-08). One of the authors (H.C.R.) is a Research Career Development Awardee of National Institutes of Health (K3-AI-6928).

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Received August 7th, 1969

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BBA 63423

On the effects of cyanide on phenoxazinone synthetase

Phenoxazinone synthetase, an enzyme which is believed to be involved in actinomycin biosynthesis, has been purified from *Streptomyces antibioticus* and studied by KATZ AND WEISSBACH[1]. These workers were unable to demonstrate a cofactor requirement of the enzyme, but they showed that it was affected by various metal ions and chelating agents. An interesting observation was that Cu^{2+} and diethyldithiocarbamate were inhibitory at low levels ($1 \cdot 10^{-4}$ and $1 \cdot 10^{-5}$ M). This suggested to us that phenoxazinone synthetase might be a copper enzyme. The fact that phe-