ON THE REVERSIBILITY BY TREATMENT WITH UREA OF THE THERMAL INACTIVATION OF E. COLI β-GALACTOSIDASE

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It is known that several enzymes can recover activity after denaturation by treatment with high concentration of urea. This has been demonstrated for a number of enzymes (Anfinsen, 1956) and recently for ribonuclease (White, 1960), α-amylase (Takagi and Isemura, 1962) and β-galactosidase (Zipser, 1963).

The exact nature of the forces involved in the denaturation of proteins by urea is still controversial, but it is known to lead to a considerable disruption of the secondary and tertiary structures and a partial unfolding of the polypeptide chains (Kauzmann, 1959).

It was thought interesting to test whether an enzyme first denatured by another means could, after being streched out by urea denaturation, recover its "natural" or active configuration.

Crystalline β-galactosidase from E. coli K12 3300 was dissolved in Tris-acetate buffer 0.1 M, pH 7.7, with 1% β-mercapto-ethanol (0.14 M), Mg^{++} 0.01 M and Mg^{++} titriplex (Merck) 0.001 M.

It was then put in a boiling water bath for 10 minutes. Denaturation of the protein was indicated by extensive clotting and total loss of enzymatic activity. The protein suspension was then dialyzed against

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an 8 molar solution of urea in the same buffer for 12 hours at 4°C, when it dissolved completely. It was then dialyzed again against the initial buffer also at 4°C for 12 hours. The protein remained in solution and 14% of the initial enzymatic activity was recovered. This activity increased to 30% after incubation at 28°C for 12 hours (Table I).

TABLE I

	Activity in enzyme units per ml at time zero	Activity in enzyme/ml after 12 hours at 28°C
Initial enzyme solution	250 000	232 000
Boiled enzyme solution	0.2	
Boiled enzyme dialyzed against urea and then buffer	35 000	75 000

In another experiment, one step dilution of the urea was compared to dialysis. One sample being diluted ten fold in buffer from 8 M to 0.8 M urea and another dialyzed against 0.8 M urea.

At time 0 after dilution, the activity recovered is 0; it increases to about 2% of the initial value (corrected for dilution) at 85 minutes and levels off. The dialyzed sample recovers about 23% of the initial activity.

The effect of denaturation by guanidine was compared to that of urea. The same boiled preparation was dialyzed against a 6 molar solution of guanidine hydrochloride in the same buffer as above, neutralized to pH 7.5 with NaOH. The protein was solubilized completely. One sample was then dialyzed against buffer to remove the guanidine; it reprecipitated and very little activity was recovered. Another sample, after being dialyzed against guanidine like the first, was dialyzed against urea and then buf-

fer, it recovered about 8% of the initial activity (Table II).

TABLE II

	Activity in enzyme units/ml
Initial enzyme solution	1 360 000
Boiled enzyme solution	0.03
Boiled enzyme dialyzed against 8 M urea diluted 10 fold in buffer (Activity x 10)	0 (at t = 0) 20 000 (at t = 85 min.)
Boiled enzyme dialyzed against 8 M urea, then 0.8 M urea	320 000
Boiled enzyme dialyzed against 6 M guanidine, then buffer	5
Boiled enzyme dialyzed against 6 M guanidine, then 8 M urea, then buffer	115 000

These results are in agreement with the postulate that the so-called native configuration of the proteins correspond to a state of maximal stability and may therefore be entirely differentiated by the primary structure as assumed by Crick (1958). The renaturation by urea treatment of heat denatured inactive insoluble enzyme appears to demonstrate the fact that the "irreversibility" of a denatured state may not be due to the lesser thermal stability of the native state, but rather to a "freezing" of the denatured state by E.G. formation of illegitimate intermolecular bounds. According to this interpretation, renaturation of heat denatured enzyme by treatment by urea followed by dialysis would be closely comparable to renaturation of DNA by annealing at intermediate temperatures.

The fast removal of urea by dilution may not allow time for the

proper bonds to be formed in the proper order. This is analogous to the non renaturation of DNA after fast cooling. Dialysis out of guanidine which is a highly ionic denaturing agent probably also allows illegitimate bonds to be formed, giving rise to intermolecular links and insoluble agregates.

The relative inefficiency of the renaturation by urea after treatment with guanidine may be due either to the partial rupture of some structure which was maintained during boiling and urea treatment or to the formation of stable bonds which may not be reversible under the conditions employed.

The increase in activity after incubation at 28°C is probably due to the necessity of polymerization of the inactive monomers into active tetramers (Zipser, 1963).

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