Resolution of Temperature-Dependent Conformers of Histidine Ammonia-Lyase on Disc Gel Electrophoresis: Correlation with Arrhenius Discontinuities

Several cases of enzymes which display discontinuous Arrhenius plots have been documented[1–5] but little evidence of the cause has been presented. Kinetic studies of *Pseudomonas* histidine ammonia-lyase[6] have provided indirect evidence that the enzyme undergoes a similar temperature-dependent transition. Histidine ammonia-lyase from *Pseudomonas* exists as a monomer and various polymeric forms[7,8]. In the present communication we shall show that temperature-dependent conformers of monomeric histidine ammonia-lyase can be resolved on a polyacrylamide disc gel, and that the presence of these conformers can be correlated with discontinuities in Arrhenius plots of kinetic data obtained with, or in the absence of, thiols.

Histidine ammonia-lyase from *Pseudomonas putida* (ATCC 12633) was purified and assayed as previously described[6]. Disc gel electrophoresis was carried out in 7.5% polyacrylamide gels at pH 9.5 according to BUCHLER[9]. When untreated enzyme is subjected to disc gel electrophoresis below 5 °C a single monomer species (about 95% of the total enzyme activity in our preparations) with an apparent mobility of 0.4, and various polymeric species[7,8] (mobility $\leq$ 0.2) are observed (Figure 1a).

When enzyme is subjected to electrophoresis at 35–40 °C a second form arises from the otherwise homogeneous monomer species (Figure 1b). The interconversion was shown to be fully reversible. When enzyme is incubated at 35–40 °C and then subjected to electrophoresis at 0–5 °C after slow cooling, the band is not split[10]. The presence of this split band is interpreted as direct evidence of the existence of two conformers of histidine ammonia-lyase. The fact that this heterogeneity is observed at the same temperature (about 35 °C) as the break in the Arrhenius plot and VAN'T HOFF plot of substrate binding[6] is certainly consistent.

If the enzyme was incubated with 5 m*M* dithiothreitol (DTT) and subjected to electrophoresis a single band was observed (Figure 1c). With glutathione (GSH) present (Figure 1d) 2 bands were observed, and they appeared to be separated more distinctly than when no thiol was present.

When Arrhenius plots were constructed from kinetic data a striking coincidence between these curves (Figure 2) and the electrophoresis results is observed. It is interesting to note that the linear, nondiscontinuous Arrhenius plot of data obtained with DTT present corresponds to a single band on electrophoresis, whereas the split band is

Correlation between Arrhenius plots and electrophoresis of histidine ammonia-lyase

Addition	Number of bands [a]	$\bar{E}_{cat}$(kcal/mole)	
		above 35 °C	below 30 °C
None	2	6 [b]	12
DTT	1	12 [c]	12
GSH	2	9	12

[a] Monomer, 35–40 °C. [b] Determined from the tangent at the highest 2 temperatures. [c] Linear Arrhenius plot.

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[10] It should be noted that in order to obtain such a separation, interconversion of conformers must be a slow process on the gel column.

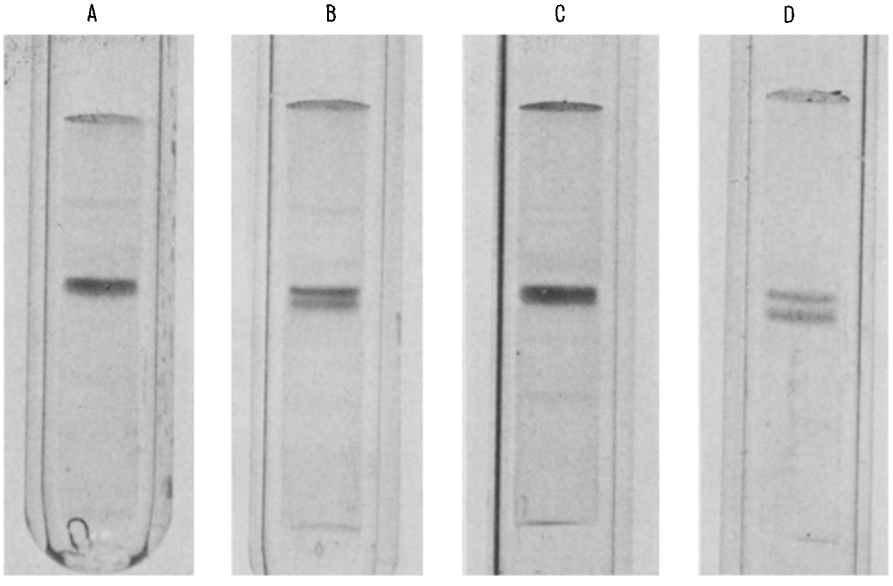

Fig. 1. Sample polyacrylamide disc electrophoresis gels run at pH 9.5. A) Below 5 °C, control; B) 35–40 °C, control; C) 35–40 °C, 5 m*M* DTT; D) 35–40 °C, 5 m*M* GSH. Gels were stained with amido black. Electrophoresis went from top to bottom.

correlated with a break in the Arrhenius plot. A summary of this correlation is shown in the Table. Since histidine ammonia-lyase contains 1 cysteinyl residue per subunit[11] and this –SH group has been implicated in the binding of substrate and catalysis[12] it seems reasonable to speculate that GSH and DTT may act in different ways on the enzyme. One model which is consistent with this data is that DTT acts solely to maintain the reduced form of the enzyme whereas GSH also forms a mixed disulfide accounting for the 2 bands observed on electrophoresis and the $E_{cat}^{\pm}$ value intermediate between those obtained in the presence and absence of DTT. This may be analogous to the situation reported by ROUFAGALIS et al.[4] concerning the binding of ligands to acetylcholinesterase.

The present study with histidine ammonia-lyase provides direct evidence that the concept[2] of a temperature-dependent equilibrium between 2 forms of enzyme is the best explanation for the phenomena reported in the literature concerning discontinuous Arrhenius plots and related thermodynamic and spectroscopic data. The physiological significance of such phenomena is a matter of uncertainty and contention. It is noteworthy, however, that *Pseudomonas* and other bacteria grow well over a wide range of temperature and that at 37 °C both conformers of histidine ammonia-lyase would exist. Such transitions have also been described for more complex structures such as myosin-ATPase[3] and membrane-bound yeast mitochondrial ATPase[13].

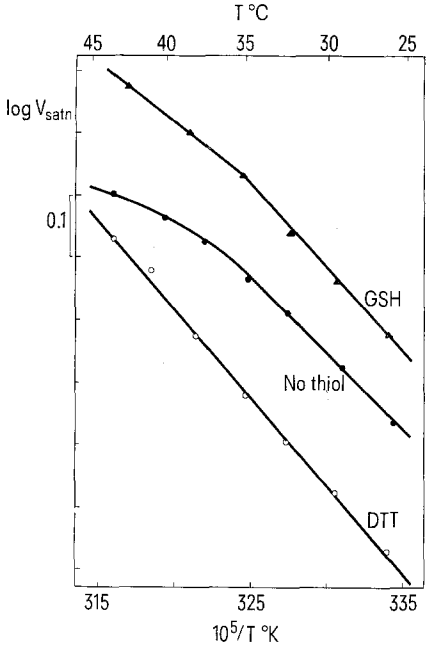

Fig. 2. Arrhenius plots of the histidine ammonia-lyase reaction. Dashes on the ordinate are spaced by 0.1 $\log_{10}$ unit. Height of curbes is merely a convenience and need not imply relative activity. Conditions are: ●, 10 DTT; ▲, 5 m*M* GSH, and ■, no thiol.

Zusammenfassung. Erstmaliger Nachweis, dass bei der *Pseudomonas* Histidin-Ammoniak-Lyase und bei Temperaturerhöhung der Enzymlösung auf 35–40° (reversibel) eine zweite Enzymspecies (Konformeres) entsteht (Disc-Gel) und dass die Umwandlung in dieser Species auch in der Arrhenius-Darstellung sichtbar ist.

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On the Nature of Unretarded Protein in a Chain Separation Method of Hemoglobin

Some years ago CLEGG *et al.*[1] introduced a new method for the separation of the α- and β-chains of human hemoglobin. This method was an improvement on former procedures and has since been used extensively by many authors[2–6].

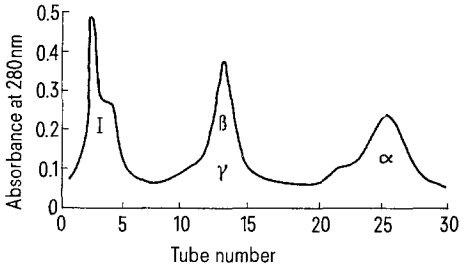

Fig. 1. Elution pattern of separation of globin chains on a carboxymethylcellulose column.

The separation is performed on a carboxymethylcellulose column in 8 *M* urea, 50 m*M* phosphate buffer pH 6.7 with 50 m*M* mercapto-ethanol. The column is eluted with a 5 to 30 m*M* Na^+-ion gradient. A representative elution pattern is shown in Figure 1. The major peaks were identified by CLEGG by the fingerprinting technique as being β- and α-chains. The small shoulders eluted just ahead of the major peaks were shown to

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