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# Enthalpy of Denaturation of Chymotrypsinogen A in Aqueous Urea Solutions

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Urea has been known as a strong denaturant for globular proteins. Numerous papers have been published in which the denaturing action of urea is described and attempts have been made to explain this action. Appropriate models have also been developed in order to calculate or at least estimate the difference in free enthalpy ($\Delta$ G) between the native and denatured forms of protein molecules in urea solutions. For a number of proteins, *e. g.*, $\beta$-lactoglobulin, $\Delta$ G's for urea denaturation at different temperatures have been obtained by optical methods, *e. g.* difference spectroscopy or optical rotatory dispersion, and from them van't Hoff's enthalpy. For a detailed survey, the reader is referred to the review article of Tanford[1].

The purpose of this communication is to report calorimetric measurements of the enthalpy of denaturation of chymotrypsinogen A in aqueous urea solution. To the authors' knowledge, this has been the first attempt to determine this enthalpy for a protein directly.

We have used a sensitive adiabatic twin calorimeter which permits measurements of relatively fast reactions (10—15 min.). The temperature difference is measured with a pair of matched thermistors which represent two arms of a Wheatstone bridge. The sensitivity of this set up was in most cases $3 \times 10^{-5}$ deg. per mm. of the galvanometer deflection. Originally, we intended to mix aqueous solutions of proteins in one vessel, and water in the other, with appropriate urea solutions. However, enthalpies of dilution of urea are relatively large and therefore we usually had to mix two urea solutions in each vessel. The mixing ratio was one (diluted urea plus protein) to four (concentrated urea). Before mixing, the solutions were thermally equilibrated in the calorimeter for about 10 hours. The temperature in all measurements was 25 °C. The chymotrypsinogen used was a 3x crystallized sample from Worthington Biochemical Corp. The initial *pH* of protein solutions was around 6.0.

The values of enthalpy differences ($\Delta$ H) for transitions between different urea concentrations obtained in our experiments are given in Table I. The values have been corrected for enthalpies of protein dilution so that they all refer to the initial concentration of the protein which was 1.2 per cent. For each value of $\Delta$ H in Table I, the initial and final concentrations of urea are given.

A detailed interpretation of the results obtained is not possible without knowing the kinetics of this denaturation and the amount of bound urea molecules at single urea concentrations. Both studies are in progress in our laboratory. However, inspection of Table I shows that urea molecules must be more

TABLE I

*Transition Enthalpies of Chymotrypsinogen A in Aqueous Urea Solutions at 25 °C*

| Urea conc. (*M*) | | — Δ H | |
|---|---|---|---|
| Initial | Final | cal. $g.^{-1}$ | kcal. $mole^{-1}$ |
| 0 | 1 | 0.66 | 16.9 |
| 0 | 2 | 1.36 | 35.0 |
| 2 | 3 | 0.50 | 12.8 |
| 2 | 4 | 1.03 | 26.5 |
| 2 | 6 | 3.12 | 80.2 |
| 2 | 8 | 5.13 | 131.8 |
| 4 | 6 | 1.71 | 43.9 |
| 4 | 8 | 3.07 | 78.9 |
| 6 | 8 | 2.03 | 52.2 |
| 8 | 9.6 | 1.82 | 46.8 |

or less continuously bound to protein polypeptide chains[2] and that the largest contribution to Δ H stems from this binding. In addition, owing to the fact that in the concentration range from 3 to 7 *M* urea the protein is only partially denatured and unfolding proceeds with a rate depending on urea concentration[1], differences in Δ H must appear between transitions proceeding directly, *i. e.*, in one step, and those proceeding in several steps. The most striking example is the difference between Δ H for transitions $2 \rightarrow 8$ (one step) and $2 \rightarrow 4$, $4 \rightarrow 8$ (two steps). This difference is — 1.03 cal. $g.^{-1}$ In the second case, 4 *M* urea is first the final solution, and then the initial solution. As has been mentioned above, we were able to measure heats evolved in a relatively short time. Therefore it may be assumed that reaction $2 \rightarrow 4$ has not come to equilibrium yet. However, when 4 *M* urea is the initial solution, this means that it was prepared at least 10 hours before mixing with the more concentrated urea solution during which time it may have come close to equilibrium. This also illustrates the importance of knowing the kinetics of this denaturation.

Regardless of this, it is possible to discuss in some detail the value of Δ H for the (one step) $2 \rightarrow 8$ *M* transition. We know that in 2 *M* urea the protein is in the native state, and in 8 *M* urea it is fully denatured[3]. This transition which is actually denaturation in urea is also very fast[3]. The value of Δ H for this transition, *cf.* Table I, is — 5.1 cal. $g.^{-1}$ or — 131.8 kcal. $mole^{-1}$ (M. W. = 25,700). The value is much more negative than expected[1] and reflects the relatively large binding of urea molecules to the protein[2].

The discussion of other values of Δ H as well as a more detailed interpretation of urea denaturation will be presented when the kinetic and binding studies will have been concluded.

REFERENCES

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## IZVLEČEK

**Denaturacijska entalpija kimotripsinogena A v koncentriranih raztopinah sečnine**

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Za merjenje toplotnih efektov, ki so zvezani z denaturacijo kimotripsinogena A v koncentriranih raztopinah sečnine, smo uporabili adiabatični kalorimeter z dvema posodama. Dobljeni rezultati kažejo, da prispeva največ k denaturacijski entalpiji vezanje molekul sečnine na proteinske polipeptidne verige. Zdi se, da se molekule sečnine vežejo na protein od vsega začetka, tj. od najnižjih koncentraciji sečnine dalje. Za termodinamsko analizo tega vezanja oz. te denaturacije sploh je potrebno imeti kinetične podatke o tej denaturaciji in množino vezanih molekul sečnine.


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