A CALORIMETRIC STUDY OF GUANIDINE HYDROCHLORIDE BINDING TO LYSOZYME

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1. Introduction

In a previous paper [1] we reported data on enthalpy changes accompanying transfer of lysozyme (EC 3.2.1.17) from one guanidine hydrochloride solution to another at 25.0°. The changes have been found to reflect changes of denaturant binding to protein with denaturant concentration. We have also given an approximate value, −300 kcal per mole of lysozyme, for the overall enthalpy of denaturation at the midpoint of unfolding at constant guanidine hydrochloride concentration, pH and temperature. The value is much higher than that for chymotrypsinogen A in urea, −70 kcal per mole of protein [2]. We decided therefore to repeat the measurements at a higher protein concentration at 25.0° and perform also measurements at 30.0°. The latter would give us a clue on how binding is influenced by temperature. Furthermore, the value of the overall enthalpy of denaturation should not be much different from that at 25.0° since the heat capacity change accompanying the transition from native state to random coil is a few kcal/degree/mole [3, 4].

2. Materials and methods

Salt-free egg-white lysozyme (6 × cryst., Lot 7102) was supplied by Miles Laboratory Inc. Ultra pure guanidine hydrochloride was purchased from Schwarz/Mann. The molecular weight of lysozyme was taken to be 14,300.

Calorimetric experiments were performed in a LKB Batch Microcalorimeter 10700-2. Initial protein concentrations were around 3% w/v, pH 5.6. Preparation of solutions and calorimetric procedure were the same as described previously [1]. The two compartments in the reaction cell were filled with 2.00 ml of 3% lysozyme in guanidine hydrochloride solution and 4.00 ml of a more concentrated guanidine hydrochloride solution, respectively, so that upon mixing a definite final concentration was obtained. The two compartments in the reference cell were filled with 2.00 ml of guanidine hydrochloride solution without protein and 4.00 ml of the same more concentrated solution. However, in this study a separate blank experiment was performed regularly for each transfer: The reaction and reference cells were filled with guanidine hydrochloride solutions of different concentration. Since the two cells were thermally not balanced – their thermal response differed for about 9% – an apparent heat effect was registered which was accounted for in the true experiment. For each initial guanidine hydrochloride concentration two experiments were carried out. Special attention was paid to the 3.0 (2.8) to 6 M transfer which leads to complete unfolding. For that transfer two blank and three actual experiments were performed. No measurements were made on solutions having initial concentrations higher than 3.0 (2.8) M and lower than 6 M in order to avoid complications with kinetics of unfolding [5].

Heat effects observed in blank experiments were of the same order of magnitude as those obtained in true experiments. This is due to the fact that heats of dilution of guanidine hydrochloride are quite large and the two calorimeter cells were thermally unbalanced, cf. above. Without performing separate blank experiments, completely erroneous values of transfer enthalpies would have been obtained.

The relative error of single determinations estimated

on the basis of results obtained in duplicate experiments was 3–5%.

3. Results and discussion

The values of transfer enthalpies obtained from calorimetric experiments after appropriate correction are assembled in table 1. Comparison of the values for 25.0° with those reported previously [1] shows that the present values are indeed substantially smaller, i.e. less negative, the difference being especially large for the 3.0 to 6 M transfer. This disagreement is in all probability due to the fact that the two cells in the calorimeter used in the previous study were also thermally unbalanced and, since separate blank experiments were not performed, the apparent heat effect resulting from that was not corrected for.

Table 1
Transfer enthalpies of lysozyme in aqueous guanidine hydrochloride solutions at 25.0° and 30.0° and pH 5.6.

Guanidine hydrochloride (M)		Temp. (°C)	ΔH (−kcal/mole)
Initial	Final		
0	1.0	25.0	25.4
		30.0	22.6
1.0	2.0	25.0	16.1
		30.0	14.9
2.0	3.0	25.0	10.8
		30.0	10.0
3.0 (2.8)*	6.0	25.0	18.2
		30.0	12.3
6.0	7.0	25.0	10.2
		30.0	10.1
7.0	8.0	25.0	8.1
		30.0	8.0

* The value in parenthesis refers to 30.0°.

Examination of the values in table 1 shows that it is not feasible to plot enthalpy values versus guanidine hydrochloride concentration in order to obtain an overall enthalpy value at the midpoint of unfolding.

However, from the data in table 1 it can also be inferred that enthalpies of transfer in the 0–3.0 (2.8) M range, where the protein from the conformational point of view is in the native state, decrease rapidly with guanidine hydrochloride concentration. The same trend is observed in the 6–8 M concentration range where the protein is completely unfolded. The enthalpy of 2–3 M transfer at 25.0° as well as at 30.0° on the other hand is seen, cf. table 1, to be nearly the same as that for 6–7 M transfer. This finding, in our opinion, reflects the fact that binding of guanidine hydrochloride to the unfolded protein is larger than to the native one [6]. In other words, were it possible to perform a binding study of the two forms over the concentration range 0–8 M one would observe higher binding to the unfolded form throughout the whole range. Higher binding yields larger heat effects. It has been claimed before [1], that the difference in solvation between the native and unfolded forms, respectively, is the sign determining factor for the overall denaturant enthalpy at constant guanidine hydrochloride concentration, pH and temperature. However, on the basis of our new findings we have to modify the above statement as follows: The overall enthalpy of denaturation under the above stated conditions is a function of denaturant concentration. Thus in the 3–6 M concentration range, e.g., the overall enthalpy becomes less negative and near 6 M it may eventually change sign and become positive. This would naturally be due to the fact that the conformational enthalpy change in this temperature range is positive and very likely does not depend on denaturant concentration [5]. So, we again arrive at the conclusion that without knowing the state of protein solvation at single denaturant concentrations in addition to knowing equilibrium data at those concentrations any detailed discussion is pointless.

The results obtained in this investigation also permit an estimate of the heat capacity change for the 3–6 M transfer. The value is 1.2 kcal/degree/mole which is about one half of the estimated conformational heat capacity change [3]. Inspection of the data in table 1 shows that the difference appears to be due mainly to smaller enthalpies of solvation at 30.0° as compared with those at 25.0° which apparently reflects smaller denaturant binding at the higher temperature.

In order to obtain additional support for the views expressed above, we intend to perform calorimetric experiments at one or two more temperatures, whereas a separate study of the denaturant binding to lysozyme is being carried out.

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