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ENERGETICS OF PROTEIN-CYCLODEXTRIN INTERACTIONS

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ABSTRACT

The energetics of interaction of a range of cyclodextrins with folded and unfolded proteins has been examined by sensitive microcalorimetry techniques. Weak interaction with exposed amino acid residues promotes unfolding and dissociation of proteins. The possibility that such interactions may facilitate the use of cyclodextrins as "chaperone-mimics" in the refolding of denatured protein has been explored with the enzyme phosphoglycerate kinase. Up to 40% regain of activity can be achieved in some cases.

1. INTRODUCTION

The interaction of cyclodextrins with amino acid groups on proteins can have several consequences. Firstly, binding of cyclodextrins to exposed side-chains on unfolded polypeptides will de-stabilise the native folded form of the protein and lead to denaturation at lower temperatures [1,2]. Alternatively, interactions with groups on oligomeric folded proteins can lead to dissociation of these protein aggregates, especially if the complexation occurs at sites in the protein-protein interface [3]. Thirdly, combining these effects, cyclodextrin interaction with unfolded proteins may enhance the solubility of denatured protein by masking the exposed hydrophobic residues, thereby possibly assisting the refolding of the polypeptide. In this way cyclodextrins might act as small chaperone-mimics in the protein folding process in cases where re-folding is inhibited by poorly-reversible aggregation or entanglement. The energetics of all these processes have been examined by sensitive differential scanning (DSC) and isothermal titration microcalorimetry (ITC) yielding information on the thermodynamics and stoichiometry of protein-cyclodextrin interactions in various systems. The effects of cyclodextrins on regain of enzyme activity following thermal denaturation are also described here.

2. MATERIALS AND METHODS

Calorimetric measurements of protein stability and dissociation energetics were done using Microcal MC2-D and Omega titration calorimeters respectively, following standard procedures [1,2]. Proteins, enzymes and cyclodextrins were obtained from Sigma or Aldrich chemical companies, as appropriate.

3. RESULTS AND DISCUSSION

3.1 Thermal Stability

The thermal stability of a range of globular proteins in solution is reduced in the presence of cyclodextrins. DSC experiments (Fig.1) show that the energetics of this process are consistent with binding of cyclodextrins to the unfolded form of the protein.

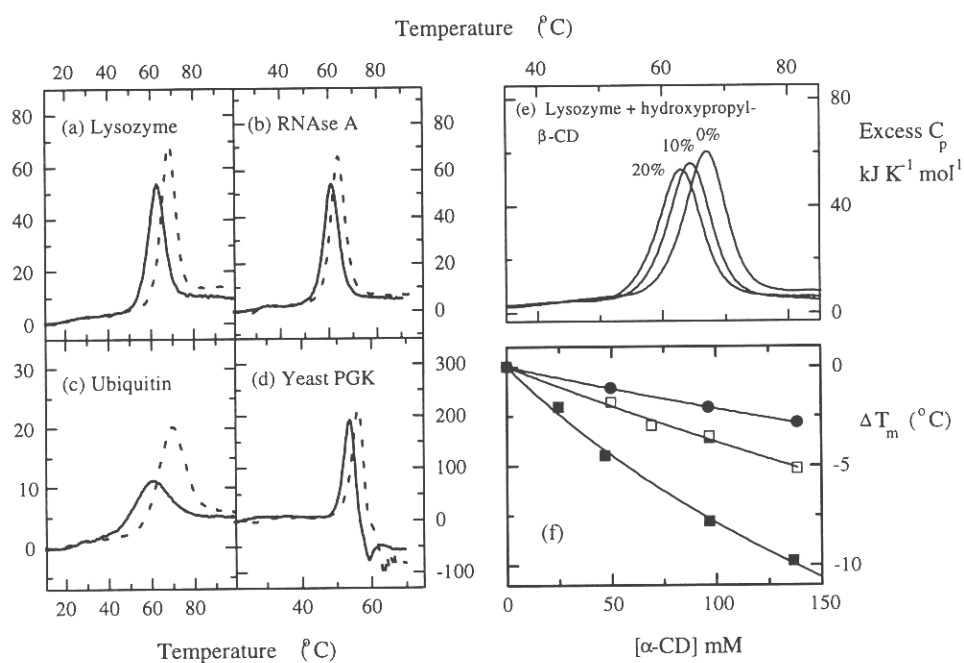


Fig.1 Effects of cyclodextrins on thermal unfolding of proteins. (a)-(d) DSC data showing excess heat capacity (ΔC_p) of various proteins in buffer alone (dotted) or in the presence of ca.100mM α -cyclodextrin. (e) Effect of increasing concentrations of hydroxypropyl- β -CD on lysozyme (pH 3). (f) Variation of thermal unfolding temperature (ΔT_m) with α -CD concentration for RNase (filled circles), lysozyme (open squares), and ubiquitin (filled squares). Curves show theoretical fits to the model described in the text.

The data can be analysed to give both the enthalpy of unfolding of the protein and the binding affinity of cyclodextrins for groups on the unfolded polypeptide. Assuming a simple unfolding equilibrium:



with cyclodextrin binding to n identical sites on the unfolded protein, the decrease in thermal unfolding temperature (T_m) can be written [2]:

$$\Delta T_m / T_m = -(nRT_{m0} / \Delta H_{\text{unf},0}) \cdot \ln(1 + [C]/K_C)$$

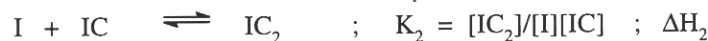
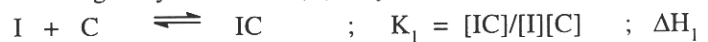
where $K_C = [C][U]/[UC]$ is the dissociation constant for cyclodextrin (C) binding to unfolded protein (U). Values obtained are compatible with known numbers of aromatic residues in these proteins ($n = 4-12$) with $\Delta H_{\text{unf},0} = 250-500 \text{ kJ mol}^{-1}$. K_C values indicate weak free energies of CD binding, with $-\Delta G^0 \approx 2-6 \text{ kJ mol}^{-1}$ at T_m .

3.2 Protein Subunit Dissociation

Interaction of cyclodextrins with amino acid residues on the surface of folded proteins will affect specific aggregation and other properties, as has been shown in the case of the insulin dimer (see accompanying paper [3] for details). This can be modelled simply as follows. Assuming simple monomer-dimer equilibrium for insulin (I), or other protein:



with sequential binding of cyclodextrins (C) only to monomer:



..... and so on for subsequent binding steps.

the apparent dissociation constant may be written:

$$K_{\text{diss}} = [I_{\text{tot}}]^2/[I_2] = K_{\text{diss},0}(1 + K_1[C] + K_1K_2[C]^2 + \dots)^2$$

Despite uncertainties regarding the number of potential binding sites and their binding affinities, this polynomial expression does form a reasonable basis for empirical modelling of the observed effects of cyclodextrins on insulin dissociation thermodynamics. The energetics of dimer dissociation have been determined by titration microcalorimetry [3] and data fit to the above model.

3.3 Protein Refolding - Chaperone Mimicry

The regain of enzyme activity after thermal denaturation of phosphoglycerate kinase (PGK) is significantly enhanced in the presence of cyclodextrins. PGK solutions (pH 7.5, 1mM DTT) were heated to 60°C for 10 min. then cooled to room temperature for 3.5 hours prior to enzyme assay. Results (Table 1) show that increasing concentrations of various cyclodextrins can promote refolding and regain of biological function in this

normally irreversible process. Similar results are obtained regardless of whether cyclodextrins are added before or after the denaturation step. Unfolded protein is sticky stuff, and PGK normally forms entangled aggregates or precipitates when thermally unfolded, as indicated by the noisy exothermic post-transition DSC baseline in Fig.1(d). Complexation of cyclodextrins with exposed groups on the unfolded protein will enhance solubility and encourage refolding rather than non-specific aggregation. In this way, cyclodextrins may mimic naturally occurring chaperone molecules that perform similar functions *in vivo*. It is important that the CD-protein interactions are relatively weak and reversible, otherwise removal of the cyclodextrins during refolding might be too difficult.

TABLE1: Regain of PGK activity after thermal denaturation.

% CD (w/v):	0	2	5	12
		%	recovery	
Without CD	ca.10	-	-	-
α -CD	-	21	26	38
methyl- β -CD	-	11	15	39

4. CONCLUSION

Weak interaction between cyclodextrins and protein groups can be measured by sensitive calorimetric techniques. This provides a useful probe of protein folding and subunit interactions, with potentially important biotechnology applications.

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