EFFECTS OF AN INTERCHAIN DISULFIDE BOND ON TROPOMYOSIN STRUCTURE

Differential scanning calorimetry

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1. Introduction

An interchain disulfide bond is readily formed between cysteines located at equivalent positions (190) on each of the two chains of rabbit skeletal tropomyosin (Tm) by Cu^{2+}-catalyzed air oxidation [1,2] or by Nbs_2 reaction [3]. In order to determine how this crosslink affects the structure of Tm and what relevance it may have to its function, we studied the thermal and denaturant induced unfolding profiles by spectroscopic methods [4]. Two effects of the crosslink were apparent in the unfolding profiles. A shift of the major unfolding transition to higher temperature or greater denaturant (GuHCl) concentrations and the appearance of a pretransition in the 30–45°C region or the 0–2 M GuHCl region. Previous comprehensive studies of Tm unfolding which were done in the presence of reducing agent to prevent disulfide bond formation obtained evidence that partially unfolded intermediates exist prior to the beginning of the major transition and that the appearance of dissociated subunit species coincided with the major unfolding transition (2–3 M GuHCl) [5]. More recently, evidence was also obtained that the partial unfolding involves local chain separation in the region of Cys-190, between 0 M and 2 M GuHCl, by studying the ability of the S–S bond to be formed as a function of [GuHCl] [4].

In order to obtain more direct evidence for the effects of the disulfide bond on the structure of tropomyosin we decided to use differential scanning calorimetry (DSC) to measure the transition peak temperatures and associated H values. The calorimetric results show the two effects of the crosslink previously observed by spectroscopic techniques with a clear indication of two steps in the thermal unfolding scan of S–S crosslinked tropomyosin.

2. Methods

Rabbit skeletal Tm (M_r 68 000) was prepared by the Bailey procedure and two derivatives, iodoacetamide-treated Tm, (IA-Tm) and Nbs_2-treated Tm (Nbs_2-Tm), prepared as outlined in [4] were used in this study. The two chains of IA-Tm are uncrosslinked because the SH groups are blocked with acetamide groups preventing interchain S–S bond formation. In contrast, the two chains of Nbs_2-Tm are crosslinked via S–S bonds formed between Cys-190 [3]. SDS–PAGE gels of each sample indicated that IA-Tm consisted essentially of monomeric poly-

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Abbreviations: Nbs_2, 5,5′-dithiobis(2-nitrobenzoate); GuHCl, guanidine hydrochloride; IA, iodoacetamide; SDS–PAGE, sodium dodecylsulphate polyacrylamide gel electrophoresis

peptide chains (M_r 34 000) (<10% dimer chains) and Nbs_2-Tm consisted essentially of dimeric polypeptide chains (M_r 68 000) (<10% monomer chains).

DSC scans were run on an instrument described in [6]. Matched pairs of platinum cells of 1 ml capacity containing solvent and sample were heated uniformly and continuously at 40°C/h. The off-balance temperature signal resulting from differences in heat capacity is used to supply power to an auxiliary heater which acts to null the temperature differential. The power fed to the lagging cell is monitored and through calibration the differences in heat capacity can be obtained.

3. Results and discussion

The thermal scan for the uncrosslinked sample (IA-Tm) shows a broad asymmetrical transition peaking near 45°C (fig.1). The S–S crosslinked sample (Nbs_2-Tm) shows two broad peaks, a minor pre-transition near 36°C and a major transition near 59°C. Thus, two effects of the crosslink are observed, a shift of the major transition to higher temperatures in the 40–70°C temperature region, and the appearance of a new transition in the 25–40°C region. The heat of the transition, ΔH_{cal}, obtained by integration using M_r 68 000, was approximately the same for both samples, ΔH_{cal} 200 kcal/mol.

The location of the transitions are in qualitative agreement with the previous fluorescence and circular dichroism results [4], although the peak of the major thermal transition appears shifted about 6°C to higher temperatures compared to the midpoint of the major spectroscopic transitions. This may in part be due to the difference in the way that the respective measurements were obtained, since the spectroscopic data were obtained about 15 min after the new temperature was reached, whereas the calorimetric scans were obtained by continuously raising the temperature at a rate of 40°C/h. The similarity of the ΔH_{cal} values for the two samples indicates that there was no large perturbation of the structure produced by the disulfide crosslink in agreement with the previous spectroscopic results.

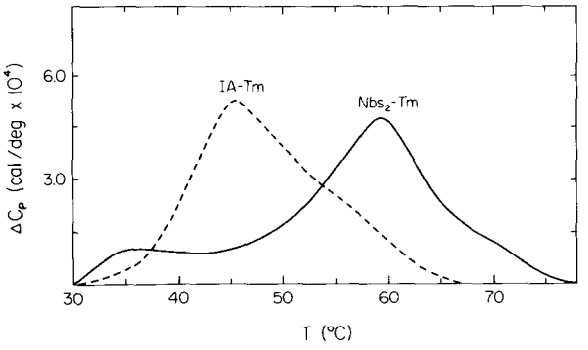

Fig.1. Differential calorimetric transitions of disulfide-crosslinked tropomyosin (Nbs_2-Tm) and uncrosslinked tropomyosin (IA-Tm). Reference cell contained 20 mM Hepes buffer, pH 7.5, 1 M NaCl; sample cell included either Nbs_2-Tm or IA-Tm at 2.5 mg/ml.

Tropomyosin is essentially an α-helical rod 400 Å long whose two subunit chains interact in register [1–3,8,9]. There are two different kinds of subunits in rabbit skeletal Tm, α and β, present in a ratio of $\alpha/\beta = 3.7$ with similar amino acid composition [10]. The α chain contains only 1 cysteine whereas the β chain contains 2 cysteines [10]. Thus, Tm from rabbit skeletal muscle is heterogeneous and consists of an approximately equal mixture of αα and αβ molecules [4,11,12] with 2 and 3 SH groups/mol, respectively. In both cases reaction with Nbs_2 results in an interchain S–S bond at Cys 190. The additional SH group on the β chain of αβ reacts normally with Nbs_2, yielding an Nbs-blocked SH group [4]. The broad DSC transitions observed for each may be due to small differences in thermal stability of the αα and αβ molecules [13]. Evidence was previously presented to indicate that the small transition observed for the crosslinked sample near 35°C, does not appear to be due to selective unfolding of only one of the cross-linked species, α–α or α–β [4].

The usual effect of a crosslink is to decrease the entropy of the denatured state, thereby increasing the relative stability of the native state. This effect is seen in fig.1 in the shift of the main transition to higher temperatures. A crosslink can also cause destabilization of the structure if energetically unfavorable contacts result from its introduction [14]. The pre-transition at 35°C can be explained if the region of the molecule near the crosslink has a relatively lower intrinsic stability. Evidence for partially unfolded intermediates has been obtained from optical rotatory dispersion and circular dichroism studies [5,13,15] and evidence

for preferential unfolding near SH groups has been obtained with ESR [16]. In addition, studies of the ability of Nbs_2 to crosslink the chains at Cys 190 showed that the chains locally separate prior to subunit dissociation [4]. Thus, the pre-transition observed in the 35°C region for the crosslinked sample may be the result of transmission of the strain across the chains resulting in greater unfolding when the chains attempt to locally separate.

Tropomyosin is thought to act as the switch in the control of actomyosin activity and contraction by moving in such a way as to unblock a myosin binding site on actin [17–19], in response to the binding of Ca^{2+} to troponin. The studies reported in this note provide further evidence that intramolecular conformational changes can occur in the physiological temperature range modified by the absence or presence of a single S–S bond. It is not known what the state of the SH groups of Tm is in vivo. Although troponin binds to the region of the molecule near Cys-190 [20], no information as yet is available to implicate reversible crosslinking in the activity of Tm.

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