# THE THERMOSTABILITY OF TROPOMYOSIN

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Tropomyosin B was recently reported to be considerably more stable to denaturation with guanidine-HCl at pH 2.0 than at pH 7.4 (Noelken and Holtzer, 1964). Riddiford and Scheraga (1962) found a similarly increased stability of paramyosin as observed by melting curves when using guanidine-urea-0.3 M KCl as solvent. This communication reports some results from a study of the conformational thermostability of tropomyosin B as estimated by specific rotation and optical rotatory dispersion measurements. We have concluded (1) that tropomyosin is more thermostable under acidic (pH 2.0) than neutral (pH 6.5, low ionic strength) or alkaline (pH 10.5) conditions, and (2) that distinct regions of the molecule are present which differ in their thermostability. Detailed results of these experiments will be published elsewhere.

We have assumed that a $b_0$ value (Moffitt and Yang, 1956) of -630 is equivalent to 100% $\alpha$-helix and that the only structural forms present in tropomyosin B are $\alpha$-helix and random coil.

Typical curves of the changes with temperature in specific rotation at 365 m$\mu$ and in helix content are shown in Figures 1 and 2. At pH 10.5, only about 60% $\alpha$-helix is present initially(10° C) and there is about 50% $\alpha$-helix at 25° C. A highly cooperative tran-

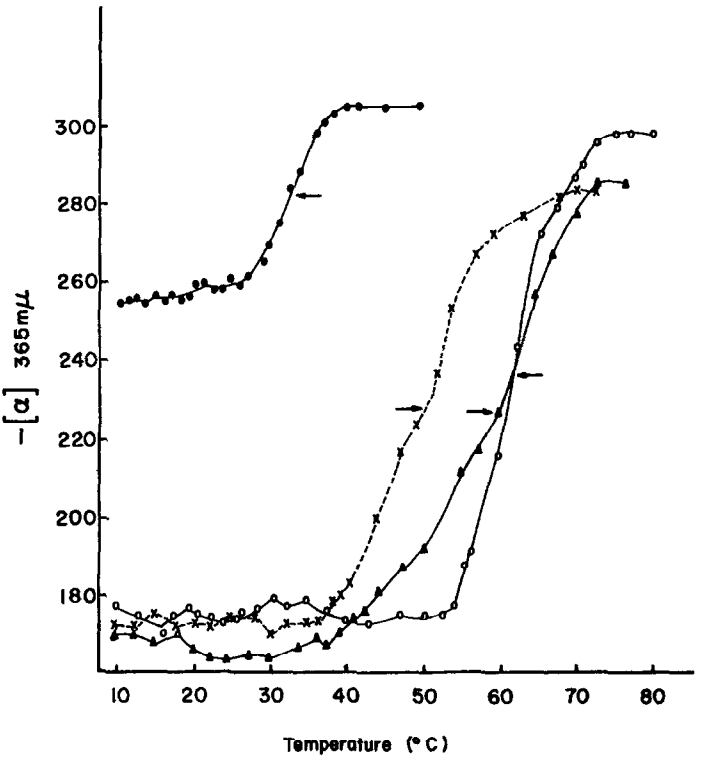


Figure 1. Change in specific rotation with temperature. • = 0.308% protein, adjusted to pH 10.5 with NaOH; 0 = 0.180% protein, pH 2.0 (0.1 M HCl); X = 0.212% protein, pH 6.5, 0.06 M phosphate buffer; ▲ = 0.210% protein, pH 6.5, 0.06 M phosphate buffer, 1.0 M KCl. Arrows indicate $t_{tr}$ (see text).

sition occurs over a narrow range of temperature (26 to 40° C, Figure 1). This transition is almost completely symmetrical with a $t_{tr}$ (the temperature at which one-half of a particular transition has occurred) of about 32.5° C.

At pH 6.5, 0.06 M phosphate buffer, $t_{tr}$ of the major transition (37-70° C) is about 50° C while the curve is skewed * by about 6° C above $t_{tr}$. At pH 6.5, 0.06 M phosphate, the presence of 1.0 M KCl shifts $t_{tr}$ of the major transition (37-72° C) upward to about 59° C.

* i. e., that half of the curve above $t_{tr}$ is broader by about 6° C than the half of the curve below $t_{tr}$.

In the latter, the skewness by some 6-7° C below $t_{tr}$ is apparent in the almost linear region (40 to 50° C) in the specific rotation curve.** At pH 6.5, with or without KCl, the width (33 to 35° C) of the major transitions suggests either a low degree of cooperativeness in the transition and/or the occurence of more than a single transition.

A loss of ~ 10% helix between 30 and 40° C and a plateau between 40 and 50° C (Figure 2) are suggested at pH 2.0. These are evident only as a slight "inverted transition" (Harrington and Schellman, 1956; Foss and Schellman, 1959) in the specific rotation curve. The major transition (52-75° C) has a $t_{tr}$ of 61° C (Figure 1) and the curve is skewed by some 4° C above $t_{tr}$. The latter may suggest the presence of more than a single transition.

A consideration of the above results leads us to conclude that different regions are present in the tropomyosin molecule which are not equally labile. At pH 10.5, three distinct regions exist. The first involves some 50% of the α-helix content which is already in the random coil conformation at 25° C (Figure 2). The second region involves 30% of the helix content which unfolds between 25 and 40° C, while the third involves the remaining 20% helix. This residual 20% helix is also present at pH 2.0 and 6.5 (Figure 2). Conversely, the two other regions distinct at pH 10.5 are not well defined at pH 2.0 and pH 6.5. In the latter cases, however, it is apparent that the 60-70% of the helix content undergoing a transformation to a random coil between 40 and 70° C includes a portion of these two regions. This conclusion is also

** The degree of skewness in the melting curve at pH 6.5 with KCl can be diminished by arbitrarily using a lower temperature for the beginning of the transition (e.g., 30° C, Figure 1). However, asymmetry in the shape of the curve would still be present.

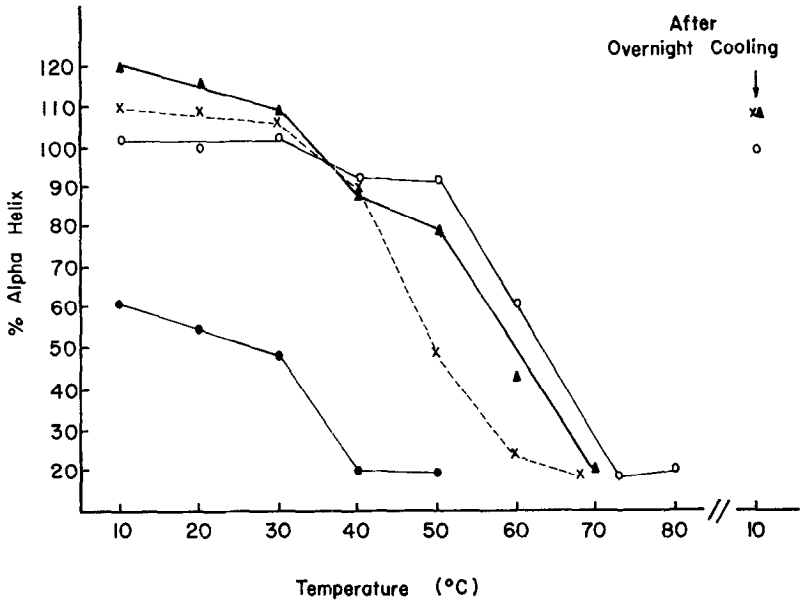


Figure 2. Change in α-helix content with temperature. ● = 0.308% protein, adjusted to pH 10.5 with NaOH; 0 = 0.180% protein; pH 2.0 (0.01 M HCl); X = 0.212% protein; pH 6.5, 0.06 M phosphate buffer, ▲ = 0.210% protein, pH 6.5, 0.06 M phosphate buffer, 1.0 M KCl.

supported by the skewness in the specific rotation curves.

The decreased helical content at room temperature at pH 10.5 (Figure 2) relative to that under neutral and acid conditions is in agreement with the findings of Lowey (1965). Also, the greater stability under acid than neutral conditions is in agreement with the results of Noelken and Holtzer (1964) with tropomyosin and paramyosin and of Riddiford and Scheraga (1962) with paramyosin. These authors suggested that carboxyl-carboxyl interactions could be responsible for the increased stability of paramyosin and tropomyosin at low pH.

A considerable increase in stability with intermediate temperatures (40 to 60° C) at pH 6.5 results from the addition of 1.0 M KCl (Figure 2). We have also observed that at lower

levels of KCl (e. g., 0.5, 0.1 M KCl) the thermostability is qualitatively proportional to the salt content. At pH 6.5, the degree of polymerization of tropomyosin is inversely proportional to the ionic strength (Tsao et. al., 1951; Doty and Sanders, 1954; Kay and Bailey, 1960; and Ooi et. al., 1962) and the monomer is present at an ionic strength of 1.1. Thus, the monomeric form appears to be most stable at pH 6.5.

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