THERMALLY INDUCED STRUCTURAL TRANSITIONS OF THE VARIABLE AND CONSTANT HALVES OF BENCE JONES PROTEINS: FLUORIMETRIC STUDIES

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SUMMARY: Fluorimetric method has been used to study the thermally induced structural transitions of the variable and constant halves of Bence Jones proteins. Like the intact protein, both the variable and constant halves exhibit significant increase in their fluorescence intensity at 50-56° due to conformational unfolding. The intact protein and its variable half, thus unfolded, exhibit considerable ANS dye (8-Anilinonapthalene-1-sulfonate) binding capacity, as measured by the increase in ANS fluorescence. However, this is not true for the unfolded constant half. The thermosolubility properties, which are shown by intact Bence Jones proteins and their variable halves but not by their constant halves, appear to correlate with the exposure of hydrophobic binding sites at elevated temperature.

Bence Jones proteins are the light polypeptide chains of different classes of human immunoglobulins and are known to exist in two different antigenic types κ or λ. These proteins exhibit unique thermosolubility property at high temperature. When heated to 55-60° near their isoelectric pH, they form precipitates which dissolve on further heating to about 90°. It seems that the formation of a precipitate is a consequence of thermally induced conformational change (1) followed by extensive aggregation (2). This conformational change also enhances the binding capacity of the protein for the dye ANS (8-anilinonapthalene-1-sulfonate) which is bound very weakly, if at all, at room temperature (3). The binding of ANS presumably occurs at hydrophobic regions of the protein and is accompanied by a large increase in ANS fluorescence quantum yield (4,5).

Amino acid sequence analyses have shown the monomeric Bence Jones protein to be composed of two halves; an N-terminal half which is characterized as the region of variable

sequences (V_L), whereas the C-terminal half has a constant sequence (C_L) specific for the same type of light chain κ or λ (6,7). It is generally accepted that the V_L region, either directly or indirectly, takes part in the antigen binding activity of the antibody molecule. Recent studies (8-10) have shown that the variable and constant halves possess, to a large extent, independent domains of secondary and tertiary structures. The halves (V_L and C_L) can be isolated by the limited proteolytic digestion of the intact protein and subsequent purifications (11, 12). In the present communication, thermally induced structural transitions of the isolated halves of Bence Jones proteins have been studied by using fluorimetric method. The purpose is to find out possible differences between the variable and constant halves with respect to such transitions which can be correlated with their thermosolubility properties.

MATERIALS AND METHODS

Preparation, isolation and characterization of the variable and constant halves of Bence Jones proteins have been described earlier (8, 13). Magnesium salt of 8-Anilinonapthalene-1-sulphonic acid (ANS) was obtained from Eastman Kodak Co. (Rochester, N.Y.) and was further purified by repeated crystallization from water. Fluorescence measurements were carried out by using an Aminco-Bowman spectrofluorimeter, equipped with a water jacketted cell holder. Water from a controlled temperature bath was circulated through the cell holder and the temperature of the solution within the cell was monitored by a telethermometer. In all cases, fluorescence was recorded immediately after the temperature equilibrium was attained. Protein concentrations were determined by the modified Folin-Ciocalteu method (14).

RESULTS AND DISCUSSION

The temperature dependent changes in the fluorescence intensity of the monomeric κ type Bence Jones protein (Ha) and its variable and constant halves are shown in Fig. 1.

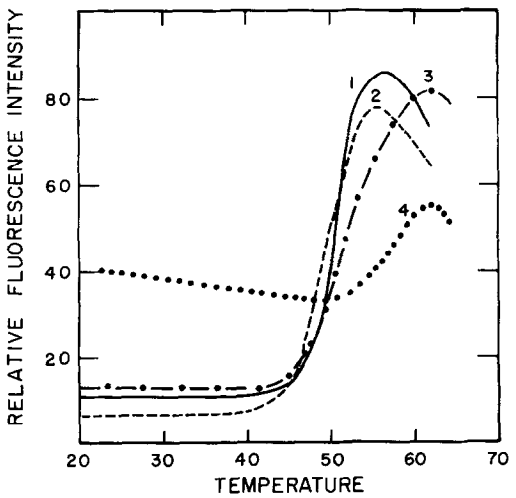

Fig. 1. Temperature dependent changes in the fluorescence intensity of a κ type Bence Jones protein (Ha) (curve 1), its variable (curve 2) and constant halves (curve 3). Also shown in the figure the same for the constant half of a λ type protein (Sc) (curve 4). Protein concentrations varied between 0.18-0.24 mg/ml. Exciting wavelength was 295 nm and the fluorescence was measured at 345 nm. Solvent used was 0.02M phosphate in 0.15M NaCl, pH 7.4.

The large enhancement in the fluorescence intensity of these proteins in the temperature range 50-56° probably reflects conformational unfolding. Similar results are also obtained with the variable and constant halves of another κ type dimeric Bence Jones protein (Ra) used in these studies. These observations are consistent with the recent circular dichroism studies (15) with different Bence Jones proteins which show that, on heating, these proteins partially unfold and tend towards a random chain structure. Similar, thermally induced, conformational unfolding has also been observed with Fab and Fc fragments of human immunoglobulin G (16). The maximum fluorescence intensity of the intact protein (Ha) and its variable half occurs almost at the same temperature (56°), whereas it is slightly higher (62°) for C_κ fragment. The maximum fluorescence intensity of the constant half of a dimeric λ type protein (Sc) occurs at the same temperature (62°), although the intensity decreases slightly between 25° and 50° (Fig. 1). Unfortunately, the V_λ fragment could not be used in these studies as it was highly aggregated at room temperature (13). The decrease in the fluorescence intensity of these proteins above about 60° may be due to thermal quenching of fluorescence at elevated temperature.

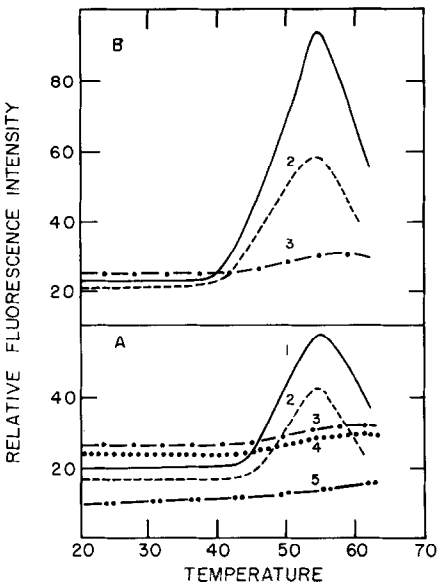

Fig. 2. Temperature dependence of the ANS binding ability of Bence Jones proteins and their variable and constant halves. (A) Changes in the fluorescence intensity of a ANS solution (25 μg/ml) containing a K type protein (Ha) (curve 1), its variable (curve 2) and constant (curve 3) halves. Curve 4 represents the same containing the constant half of a λ type protein (Sc). Curve 5 was obtained without any protein. Protein concentrations varied between 0.18-0.20 mg/ml. Exciting wavelength was 365 nm and the fluorescence was measured at 470 nm. Solvent used was 0.02 M phosphate in 0.15 M NaCl, pH 7.4. (B) Changes in the fluorescence intensity of a ANS solution (25 μg/ml) containing a K type protein (Ra) (curve 1), its variable (curve 2) and constant halves (curve 3). Protein concentrations and other conditions were the same as described above.

Although both the variable and constant halves undergo conformational unfolding at high temperature, only the variable half exhibits the thermosolubility property which is characteristic of the intact Bence Jones protein (8, 11). This suggests that there must be some structural differences between the unfolded variable and constant halves. This is clearly supported by the data in Fig. 2 where the enhancement in ANS fluorescence due to its binding to the protein has been plotted against temperature. Both the K type Bence Jones proteins (Ha and Ra) seem to bind ANS considerably at higher temperature (50-56°) and the increase in the fluorescence intensity is higher in the case of dimeric protein (Ra). The interesting observation is that the variable halves of both (Ha) and (Ra) proteins show ANS binding at high temperature (50-56°), but of a lesser magnitude than those of intact proteins. However, the constant halves of neither the K nor the λ type proteins exhibit any detectable

amount of temperature dependent ANS binding. As ANS molecule is known to exhibit strong increase in its fluorescence intensity in non polar environment (4, 5), it is likely that intact Bence Jones proteins and their variable halves on unfolding expose some hydrophobic residues which bind ANS. This type of hydrophobic binding site is presumably absent in the constant halves of unfolded κ and λ type proteins. It may be mentioned here that Parker and Osterlend (17) have shown that many specific and normal immunoglobulins and their heavy chains bind ANS appreciably at room temperature and these hydrophobic binding sites are probably the same as their antigen combining sites.

The data presented here suggest that the thermal precipitation exhibited by Bence Jones proteins and their variable halves may be due to the exposure of buried hydrophobic residues and their interaction with each other causing aggregation at isoelectric pH. The decrease in the ANS fluorescence at still higher temperature (above 56°) may be due to thermal quenching of the fluorescence of the bound dye. However, it may also be due to the weakening of the hydrophobic interaction at higher temperature which decreases the ANS binding ability of the unfolded protein. This is also consistent with the observation that above 85-90°, dissolution of the precipitate occurs due to dissociation of the aggregated product into smaller units (2).

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