EFFECT OF D_2O ON THE TEMPERATURE-DEPENDENT SOLUBILITY OF CRYOGLOBULIN AND NONCRYOGLOBULIN IgM

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Received 4 April 1977

1. Introduction

Monoclonal cryoimmunoglobulins are immunoglobulins which become reversibly insoluble at low temperatures [1]. These proteins are most often associated with various lymphoproliferative disorders. While a distinct clinical significance, including increased blood viscosity and intravascular precipitation, has been ascribed to the presence of these proteins, the reason for their abnormal solubility properties is unknown [2]. Noncryoimmunoglobulins usually maintain their solubility in the cold at concentrations exceeding 200 mg/ml, while cryoglobulins precipitate from solution at these temperatures at concentrations as low as 0.1 mg/ml. Because cryoglobulins undergo a relatively large change in solubility over a narrow range of temperature and protein concentration (frequently a 10^3–10^4-fold change in solubility over a 10°C temperature range), their cold insolubility is generally assumed to arise from a major structural defect. We show in this report that the simple substitution of D_2O for H_2O as a solvent has not only a profound effect on the cold-dependent insolubility of a monoclonal, IgM-κ cryoglobulin (McE), but also imparts varying degrees of cold-dependent insolubility to a number of monoclonal noncryoglobulin IgM proteins.

2. Materials and methods

Isolation of both cryoglobulin and noncryoglobulin IgM proteins and intrinsic fluorescence, circular dichroism, infrared and ultraviolet absorption spectroscopy of these proteins will be described elsewhere. Purified proteins were exchanged into D_2O by precipitation induced by either low temperature (cryoglobulin) or low ionic strength (noncryoglobulin) and subsequent redissolution into D_2O containing 0.15 M NaCl, pH 8.0. This process was repeated three times to produce stock protein solutions of approximately 10 mg/ml. Cryoprecipitation was assayed by dilution of stock solutions with isotonic D_2O. The effect of temperature was studied employing a temperature-regulated Sorvall RC2B ultracentrifuge.

3. Results and discussion

The effect of protein concentration upon the cold-induced insolubility of cryoglobulin McE and three noncryoglobulin IgM proteins in isotonic D_2O is illustrated in fig.1. For comparison purposes, the

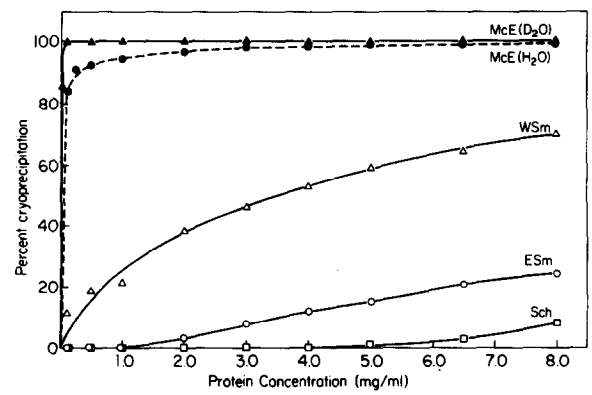

Fig.1. Effect of protein concentration on the cryoprecipitation of cryoimmunoglobulin McE in H_2O and D_2O, and on the solubility at 2°C of the noncryoglobulin IgM proteins WSm, ESm and Sch in D_2O. All of the solutions also contained 0.15 M NaCl, pH or pD 8.0.

concentration dependence of McE in isotonic H_2O is also shown. An enhancement of cryoprecipitation by the cryoglobulin is observed in D_2O solution. D_2O also appears to induce varying degrees of cold-dependent insolubility among a number of other noncryoglobulin IgM molecules. Of the five monoclonal, noncryoglobulin IgMs we examined, three displayed detectable cold-dependent insolubility in D_2O (fig.1). This effect was detectable at D_2O concentrations as low as 10% with the noncryoglobulin WSm and 20% with the other noncryoimmunoglobulins, with a roughly linear correlation between the concentration of D_2O and the magnitude of the effect upon the cold-induced insolubility. The enhancement of cryoprecipitation of McE showed a similar dependence on D_2O concentration. We estimate the solubilities of these three noncryoglobulin proteins in isotonic H_2O at 2°C to be in excess of 200 mg/ml. To further explore these effects, the temperature dependence of their insolubilization was investigated (fig.2). The enhancement of cryoprecipitation of McE in D_2O is seen to be accompanied by a significant increase in the temperature at which the insolubility occurs. The midpoint at which 50% cryoprecipitation appears is shifted from 11–22°C. A shift of 2°C in the midpoint was evident with D_2O

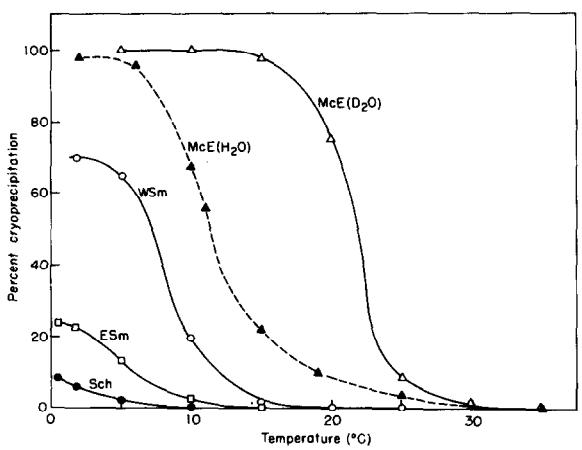

Fig.2. Effect of temperature on the cryoprecipitation of cryoimmunoglobulin McE in H_2O and D_2O, and on the solubility of the noncryoglobulin IgM proteins WSm, ESm and Sch in D_2O. All of the solutions also contained 0.15 M NaCl, pH or pD 8.0; McE concentration ~ 2.5 mg/ml; WSm, ESm and Sch concentrations ~ 8.0 mg/ml.

concentration as low as 5%. The cold-dependent insolubility occurs at much lower temperatures for the three noncryoglobulin IgM proteins, but the temperature dependence is qualitatively similar to that displayed by the cryoimmunoglobulin.

The D_2O could be altering any number of solvent-dependent properties, including either direct effects upon the conformation of the proteins or upon the intermolecular and solute–solvent interactions responsible for cryoprecipitation (or both). Examination of the proteins in both pure isotonic H_2O and D_2O and in mixtures of the two solvents employing circular dichroism, intrinsic fluorescence, near and far ultraviolet absorption and infrared spectroscopy produced no evidence for alterations in either the secondary or tertiary structure of any of the IgM molecules. Therefore, detectable changes in conformation of the immunoglobulins do not appear to be responsible for the cold-dependent insolubility produced by the D_2O.

The substitution of D_2O for H_2O has been shown to have a variety of effects on noncovalent interactions between protein molecules. The presence of D_2O is usually associated with increases in polymerization and aggregative properties in self-associating systems, as well as small increases in thermostability [3–9]. Explanations offered for these observations include specific and nonspecific deuteration effects, changes in pK of ionizable groups, modification of the strength of both hydrophobic- and hydrogen-bonding interactions, and perturbations of dispersion forces [9–13]. Thus, the effects of D_2O upon cryoprecipitation could reflect the involvement of a variety of weak noncovalent interactions.

The precise significance of the D_2O-induced, cold-dependent insolubility of normal IgM molecules is unclear, but these observations do suggest that the ability of normally cold-soluble IgM proteins to maintain their solution state at low temperatures is probably marginal. It appears likely that only minor changes in protein-solvent interactions are necessary for cryoglobulin behavior to become manifest among IgM normally lacking cold-induced insolubility. It is quite possible that immunoglobulin variants possessing the cryoglobulin property could arise naturally through the mechanisms involved in generation of antibody diversity. The presence of these altered molecules would only become evident when

they are present in abnormally high concentrations, as seen in certain lymphoproliferative diseases.

Acknowledgements

This work was supported by NIH AI-13528, NIH CA-08748 and NIH CA-17404.

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