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Fluoroalcohol-induced structural changes of proteins: some aspects of cosolvent-protein interactions

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Abstract The conformational transitions of bovine \( \beta \)-lactoglobulin A and phosphoglycerate kinase from yeast induced by hexafluoroisopropanol (HFIP) and trifluoroethanol (TFE) have been studied by dynamic light scattering and circular dichroism spectroscopy in order to elucidate the potential of fluoroalcohols to bring about structural changes of proteins. Moreover, pure fluoroalcohol-water mixed solvents were investigated to prove the relation between cluster formation and the effects on proteins. The results demonstrate that cluster formation is mostly an accompanying phenomenon because important structural changes of the proteins occur well below the critical concentration of fluoroalcohol at which the formation of clusters sets in. According to our light scattering experiments, the remarkable potential of HFIP is a consequence of extensive preferential binding. Surprisingly, preferential binding seems to play a vanishing role in the case of TFE. However, the comparable Stokes radii of both proteins in the highly helical state induced by either HFIP or TFE point to a similar degree of solvation in both mixed solvents. This shows that direct binding or an indirect mechanism must be equally taken into consideration to explain the effects of alcohols on proteins. The existence of a compact helical intermediate with non-native secondary structure on the transition of \( \beta \)-lactoglobulin A from the native to the highly helical state is clearly demonstrated.

Keywords Protein folding · Light scattering · Circular dichroism · Hexafluoroisopropanol · Trifluoroethanol

Abbreviations CD: circular dichroism · DLS: dynamic light scattering · HFIP: hexafluoroisopropanol · \( \beta \)-Lg A: \( \beta \)-lactoglobulin A · PGK: phosphoglycerate kinase · TFE: trifluoroethanol

Introduction

Protein molecules adopt their native conformation in solution and within the cell only under specific environmental conditions. Studying the structural and thermodynamic response of proteins in dependence on solvent conditions is thus a pertinent way to elucidate their stability, folding pathways, and intermolecular aggregation behavior. Among different cosolvents used for this purpose, alcohols, and particularly their fluorinated derivatives, have found a wide range of applications. Alcohols mainly disrupt the native tertiary structure by weakening hydrophobic interactions and strengthening helical propensities (Thomas and Dill 1993). This special effect of alcohols as structure-inducing denaturants was already recognized several decades ago (Weber and Tanford 1958; Inoue and Timasheff 1968). Thus, alcohols are suitable to modulate the interactions between the polypeptide chain and the solvent and the interactions between different segments of the polypeptide chain as well. Therefore, fluorinated alcohols, mostly such very efficient members like trifluoroethanol (TFE) and hexafluoroisopropanol (HFIP), have been used not only in order to promote the formation of helical structure in protein fragments and peptides (Nelson and Kallenbach 1986; Sönnichsen et al. 1992; Dyson and Wright 1993; Luo and Baldwin 1997) but in many other instances such as the transformation of proteins into molten globule-like intermediates (Buck et al. 1993; Alexandrescu et al. 1994; Cort and Andersen 1997; Gast et al. 1999; Konno et al. 2000) and the
stabilization of intermediate structures of proteins (Luo and Baldwin 1998). Recent investigations have dealt with the influence of cosolvents on the folding kinetics of proteins (Lu et al. 1997; Chiti et al. 1999a; Hamada et al. 2000), the tuning of solvent conditions for studies of the amyloid formation of peptides and proteins (Barrow et al. 1992; Chiti et al. 1999b), the dissection and reassembly of amyloid fibrils (Barrow et al. 1992; MacPherson and Dobson 2000), and the $\alpha$-$\beta$ transition of proteins (Dong et al. 1998; Kuwata et al. 1998), which plays an essential role in various conformational diseases. Furthermore, fluoroalcohols are widely used in the field of peptide chemistry to dissolve peptide aggregates. A comprehensive overview concerning the effects of TFE and related cosolvents on polypeptide chains has been given by Buck (1998).

Despite the numerous applications and the various effects observed so far, the detailed mechanisms of how alcohols act on proteins and peptides are still not well understood, nor can the order of effectiveness of various alcohols in provoking conformational transitions be explained in a satisfactory manner. Obviously, their action results from a superposition of the effects of different properties (solvent polarity, dielectric constant, length of the carbohydrate chain, number of OH groups, degree of halogenation), which can hardly be separated adequately. In this respect, the consideration of the influence of these properties in terms of group additive contributions (Hirota et al. 1998) was an essential step forward in quantifying the effects of different alcohols on polypeptides. For some frequently used alcohols the order of effectiveness is HFIP $>$ TFE $>$ isopropanol $>$ ethanol $>$ methanol (Hirota et al. 1997). Nevertheless, the extremely high potential of HFIP and also TFE compared to other alcohols suggests the existence of additional factors. These alcohols will be considered in more detail. The tendency of HFIP and to a lesser extent of TFE to form clusters in aqueous solutions (Kuprin et al. 1995; Gast et al. 1999) has been proposed to be an important factor (Hirota et al. 1997, 1998; Hong et al. 1999). It is one purpose of this work to find out proper relationships between cluster formation and the effectiveness of HFIP and TFE in aqueous solutions.

Cluster formation is obviously a consequence of the partially hydrophobic nature of fluorinated alcohols. It has been proposed that the hydrophobicity of fluorinated alcohol molecules plays an essential role for attachment at the polypeptide chain, leading to their high efficiency (Rajan and Balaram 1996). An indication that hydrophobic interactions play an important role at least for some fluoroalcohols is supported by the observation of cold denaturation of the induced structures (Andersen et al. 1996; Bhattacharjya et al. 1999).

It is still a matter of dispute whether alcohols attain their effects by direct binding to the polypeptide chain (Jasanoff and Fersht 1994; Rajan and Balaram 1996; Luo and Baldwin 1997) or by an indirect mechanism caused by alcohol-mediated changes in the solvent shell around the polypeptide (Conio et al. 1970; Cammers-Goodwin et al. 1996; Walgers et al. 1998). It is conceivable that various alcohols have very different binding affinities to peptides and therefore differ strongly in the way they substitute the normal aqueous environment of the polypeptide chain. Earlier investigations, including studies of the influence of 2-chloroethanol on $\beta$-lactoglobulin A and other proteins (Timasheff and Inoue 1968; Inoue and Timasheff 1972), suggested that preferential binding of the alcoholic component and conformational changes occur in parallel fashion. It is a further important purpose of this work to relate the preferential binding of HFIP and possibly TFE to the ability to induce conformational transitions in the investigated proteins.

For our investigations, we have chosen bovine $\beta$-lactoglobulin A ($\beta$-Lg A) and phosphoglycerate kinase (PGK) from yeast to study the conformational transitions induced by an increasing volume fraction of HFIP in 10 mM HCl, pH 2. In 10 mM HCl, $\beta$-Lg A is in the monomeric compactly folded native state (Kuwata et al. 1999), while PGK attains a highly unfolded conformation under these conditions (Damaschun et al. 1998, 1999). Accordingly, these proteins represent two totally different model systems: in the first case, HFIP interacts with an originally folded protein, while in the second case the polypeptide chain is entirely exposed to the solvent. For comparison, some experiments were also performed in the presence of TFE. The changes in secondary and tertiary structure were observed using circular dichroism (CD) spectroscopy. Cluster formation, changes of the hydrodynamic dimensions, and preferential binding to the polypeptide chains were monitored by combined dynamic and static light scattering.

The aim of this work is to give further evidence of how fluoroalcohols may act on polypeptide chains. We will demonstrate that essential structural changes of both proteins appear well before the critical concentration for cluster formation of alcohol molecules is reached. Thus, cluster formation of fluoroalcohols in aqueous solutions cannot be the primary cause of the induced structural changes. Both phenomena are independent reflections of the hydrophobicity of the particular fluoroalcohol. The high efficiency of HFIP correlates with its strong preferential interaction with polypeptide chains. Furthermore, our dynamic light scattering data directly demonstrate the existence of a compact helical intermediate state during the transition of $\beta$-Lg A from the native to the expanded helical state.

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**Materials and methods**

**Materials**

HFIP (>99%, GC) and TFE (>99%, GC) were obtained from Sigma-Aldrich (Germany) and Fluka (Switzerland), respectively. The proteins, $\beta$-Lg A (bovine milk) and PGK (yeast), were purchased from Sigma-Aldrich and from Roche Diagnostics (Germany), respectively. All other chemicals were of analytical