Application of a Two-State Kinetic Model to the Heterogeneous Kinetics of Reaction between Cysteine and Hydrogen Peroxide in Amorphous Lyophiles

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ABSTRACT: The bimolecular reaction between cysteine (CSH) and hydrogen peroxide (H_2O_2) in amorphous PVP and trehalose lyophiles has been examined at different reactant and excipient concentrations and at varying pH and temperature. Initial rates of product formation and complete reactant and product concentration-time profiles were generated by HPLC analyses of reconstituted solutions of lyophiles stored for various periods of time. While only cystine (CSSC) forms in aqueous solutions, cysteine sulfinic (CSO_2H) and sulfonic (CSO_3H) acids are significant degradants in amorphous solids. The formation of alternative degradants was consistent with the solution reaction mechanism, which involves a reactive sulfenic acid (CSOH) intermediate, coupled with the restricted mobility in the amorphous solid-state, which favors reaction of CSOH with the smaller, mobility-advantaged H_2O_2 over its reaction with cysteine. Complex rate laws (i.e., deviations from 1st order for each reactant) observed in initial rate studies and biphasic concentration-time profiles in PVP were successfully fitted by a two-state kinetic model assuming two reactant populations with different reactivities. The highly reactive population forms CSSC preferentially while the less reactive population generates primarily sulfinic and sulfonic acids. Reactions in trehalose could be described by a simple one-state model. In contrast to the reaction in aqueous solutions, the 'pH' effect was minimal in amorphous solids, suggesting a change in the rate-determining step to diffusion control for the model reaction occurring in amorphous lyophiles. J Pharm Sci 97:3907–3926, 2008

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INTRODUCTION

Understanding, and ultimately controlling, the kinetics of physical and chemical degradation processes in amorphous solid pharmaceutical formulations is a topic receiving increasing attention by pharmaceutical scientists. This can be attributed in part to the expanding number of biopharmaceutical products (e.g., peptides and proteins) that are formulated in lyophilized form for reconstitution prior to their use[1,2] and to a resurgence in the development of amorphous formulations to improve bioavailability of poorly soluble compounds.[3–5] Another significant factor is the recent progress in understanding the nature of the amorphous state, the role of moisture, and the importance of molecular mobility even in glasses well below their glass transition temperatures.[6–16]

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Chemical reaction kinetics in amorphous glasses often exhibit complex rate laws and in some cases may be closely coupled to one or several underlying relaxation processes in the matrix.[17–20] In particular, bimolecular reaction rates may depend on the diffusive motion of the reactants, which are severely constrained in amorphous glasses. Whereas translation diffusion is generally sufficiently rapid in solution as to have no significant influence on reaction kinetics for most pharmaceutically relevant reactions, reactant diffusion may not occur within reasonable time frames in amorphous glasses except over very short distances or within "mobile" regions.[7] For bimolecular reactions in amorphous glasses, heterogeneous dynamics may arise from the distribution of distances that randomly diffusing reactant molecules must travel in order to react.

Glasses also relax or "age" over time toward their equilibrium states. Stillinger[21] provided a topological view of glasses in terms of local particle arrangements represented as a hypersurface of potential energy wells of varying depth separated by variable activation barriers leading to a broad distribution of relaxation times with transitions between basins separated by low activation energy barriers occurring over short periods of time while relaxation processes over higher activation barriers occur only at longer time scales. These relaxation processes are often characterized empirically by the Kohlrausch–Williams–Watts "stretched" exponential function that mathematically takes into account the observed heterogeneity in relaxation dynamics.[22–24] Whether or not chemical reactions are closely coupled to these relaxation processes may depend on the type of reaction involved and the nature of the relaxation processes.[25]

Another factor that may lead to complexity in reaction kinetics in amorphous solids is the potential existence of multiple phases or microdomains in the solid. Kinetically disordered glasses, for example, retain the molecular packing motif of their crystalline counterparts but only for very short distances.[26] Locally ordered domains in such materials are separated by relatively disordered strain field interfaces. Drug crystallization at surfaces in amorphous solids appears to occur, in some cases, at much higher rates than in the bulk amorphous material,[27] suggesting two types of domains having dramatically different reaction tendencies. Heterogeneity in terms of drug molecule distribution, water clustering, and phase separation of formulation components has also been described in amorphous glass excipients such as PVP.[16,28]

Many reactions of pharmaceutical interest involve multiple reaction steps, often with the formation of a reactive intermediate along the reaction coordinate, thus further complicating their mathematical treatment when the reaction occurs in amorphous glasses. Consideration of the degree to which each of the reaction steps may be altered by heterogeneity in reactant distribution within the solid or coupled to the underlying relaxation processes in the matrix will ultimately be necessary to understand reactivity in amorphous solids. The sensitivities of each reaction step to the various relaxation modes and time scales that can be detected in an amorphous material are likely to vary.

Drug oxidation is a common mode of degradation second only to hydrolysis in terms of its importance in pharmaceutical systems.[29,30] Proteins and peptides containing one or more cysteine residues are especially susceptible to thiol oxidation. Previous studies in our laboratory and elsewhere[31–34] have shown that in aqueous solution in the absence of catalysis by metal ions, thiols (RSH) can react with hydrogen peroxide (H_2O_2) via rate-determining thiolate anion attack to form a reactive sulfenic acid intermediate (RSOH). The reactive intermediate then combines with a second thiolate to form a disulfide (RSSR) as the final product of the reaction. More recently, we conducted an initial rate study of the kinetics of the same reaction between cysteine (CSH) and hydrogen peroxide (H_2O_2) as a function of reactant concentration at fixed pH in amorphous polyvinylpyrrolidone (PVP) in an effort to determine the degree to which an understanding of the reaction mechanism in solution could facilitate understanding of the reaction kinetics in an amorphous glass.[35] Even in the initial rate region, the amorphous solid-state reaction exhibited a more complex rate law in comparison to the solution reaction and produced multiple degradation products. Qualitatively, these results could be rationalized by invoking a reaction mechanism similar to the solution mechanism after factoring in molecular mobility considerations which favor those pathways involving the more mobile H_2O_2 molecules.

The goal of the present project was to study the chemical reactivity of the model thiol cysteine (CSH) and H_2O_2 in amorphous PVP and trehalose lyophiles over a wide range of reactant concentrations, pH, temperature, and reaction times and to