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Protein reactivity with singlet oxygen: Influence of the solvent exposure of the reactive amino acid residues

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ABSTRACT

The singlet oxygen quenching rate constants were measured for three model proteins, bovine serum albumin, β-lactoglobulin and lysozyme. The results were analyzed by comparing them with the corresponding singlet oxygen quenching rate constants for a series of tripeptides with the basic formula GlyAAGly where the central amino acid (AA) was the oxidizable amino acid, tryptophan, tyrosine, methionine and histidine. It was found that the reaction rate constant in proteins can be satisfactorily modelled by the sum of the individual contributions of the oxidizable AA residues corrected for the solvent accessible surface area (SASA) effects. The best results were obtained when the SASA of the AA residues were determined by averaging over molecular dynamics simulated trajectories of the proteins. The limits of this geometrical correction of the AA residue reactivity are also discussed.

1. Introduction

Singlet oxygen, O_2 ($^1\Delta_g$), is the lowest electronic excited state of molecular oxygen [1]. It is a non-radical reactive oxygen species (ROS) which has been extensively studied over the last decade due to its role in a number of physiological processes. Proteins are a major intracellular target for singlet oxygen due to their abundance within biological systems and their corresponding fast reaction rate constants [2,3]. The reactions of singlet oxygen with free amino acids (AA) have been well documented and it has been determined that at physiological pH only five amino acids out of the twenty natural AA have significant rate constants. These AA include the two sulphur containing AA of methionine and cysteine as well as the unsaturated AA, histidine, tyrosine and tryptophan, with the reported rate constants varying between 0.5×10^7 and 7×10^7 $dm^3\ mol^{-1}\ s^{-1}$ [2,4–10], except for protonated cysteine that has a reaction rate constant smaller than 4×10^4 $dm^3\ mol^{-1}\ s^{-1}$ [11]. These rate constants refer to the AA oxidation reaction. Additionally, tryptophan interacts with singlet oxygen via physical quenching thus regenerating ground state molecular oxygen. It has also been reported that at high pH values the amino acids of lysine and arginine also react with singlet oxygen [12]. In addition to those studies focused on the oxidation of the free AA, research has also been directed at the rate of reactions of di or tripeptides with singlet oxygen. Such work aims to determine the eventual influence of the peptide bond on the singlet oxygen mediated photooxidation of AA residues but the results reported so far are somewhat contradictory [5,13–18].

The rate constants for the reaction of singlet oxygen with proteins fall in the range 10^8–10^9 $dm^3\ mol^{-1}\ s^{-1}$ [4]. For a given protein the magnitude of the bimolecular quenching rate constant is generally assumed to be the sum of the rate constants for the interaction of the individual AA [19]. However, more recently it has been suggested that the reactivity of an AA residue in a folded protein is lower than that of a free AA. This systematic decrease in reactivity is very likely due to a lower accessibility to external reactants of the AA residue embedded in the protein structure.

Although there are numerous studies which concern the singlet oxygen quenching by AA and proteins, very few of them have considered the relation between the AA residues and their solvent exposure. Among these studies, the quenching of singlet oxygen by tryptophan within the protein of melitten [20]. In this case the tryptophan is fully exposed to the solvent and it was found that its rate constant is similar to that of the free AA. A study by Jensen et al. [21] showed that the rate constant for the reaction of tryptophan residues with $^1O_2$ can vary by a factor of 6 depending on the position of the tryptophan within the protein. More recently, Giménez et al. [22] demonstrated that singlet oxygen is able to diffuse towards BSA and is able to interact with both buried and exposed tyrosine and tryptophan residues.

In the present work we analyze the relation between the solvent exposure of the oxidizable AA residues and the reactivity with the singlet oxygen of three model proteins: bovine serum albumin (BSA), lysozyme and β-lactoglobulin (BLG). In order to separate the accessibility effects from the possible peptide bond effects, we took as reference for the AA residue reactivity the reactivity measured for four model tripeptides. These tripeptides have the general formula Gly-AA-Gly, by which the central AA are the oxidizable AA, histidine, tyrosine, tryptophan,

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phenylalanine or methionine. The corresponding tripeptide of cysteine was not considered due to its much smaller reaction rate constant and the small number of free cysteine found within the proteins studied. The solvent exposure of the AA residues in proteins was quantified by using solvent accessible surface area (SASA) calculations, and the effective oxidation rate constant of the protein was compared to the sum of the contributions coming from the reactive AA residues present in the protein sequence. In evaluating the SASA of different AA residues, we have taken into account the fact that this is a dynamic parameter that can vary during the protein motion. Consequently, the SASA values have been averaged over protein molecular dynamic (MD) trajectories of 10 ns each.

2. Experimental

2.1. Materials

GlyHisGly, GlyMetGly, GlyPheGly, GlyTrpGly and GlyTyrGly (Fig. 1) were purchased from Bachem and were used as received. Methylene blue, crystallized bovine serum albumin (≥96% fatty acid free), lysozyme (chicken egg white, ≥90%) and β-lactoglobulin (bovine milk, ≥90%) were obtained from Sigma-Aldrich and were used without further purification. D_2O (99.9% purity) was obtained from INC-DTCI-ICSI (Vâlcea, Romania) and was used as received.

2.2. Instrumentation and Methods

Kinetic measurements were made using a Nd:YAG laser operating on the second harmonic at 532 nm. Time-resolved singlet oxygen phosphorescence (Eq. (1))

$$O_2(^1\Delta_g) \rightarrow O_2\left(^3\sum_g^-\right) + h\nu(1270\ \text{nm}) \quad (1)$$

was measured by using a cooled NIR photomultiplier (Hamamatsu H-10330) whose output was fed to a digital scope (Tektronix DPO 7254). The sample was placed in a 1 cm cuvette crossed by the laser beam. The phosphorescence was collected at 90° and was heavily filtered against wavelengths other than 1270 nm by an optical arrangement (lenses, apertures and filters) placed in front of the NIR photomultiplier. Triggering of the oscilloscope run was ensured by a TTL signal from the laser source.

The rate constants (k_q) for the quenching of singlet oxygen by the tripeptides and proteins were determined in D_2O by monitoring the time resolved phosphorescence following laser excitation of methylene blue (MB) at 532 nm. This choice of wavelength precludes any absorption by the tripeptides and proteins and in all experiments the absorbance of the sensitizer at the excitation wavelength was fixed at 0.25. At each concentration of tripeptide or protein the recorded luminescence trace was obtained by signal averaging over a thousand single shots. The averaged traces were fitted using a single exponential. Linear plots of tripeptide or protein concentration versus the observed pseudo first order rate constant, k_2, for singlet oxygen deactivation were obtained, where $k_2 = k_1 + k_q[Q]$, k_1 being the intrinsic first order decay constant in the absence of the quencher. The photosensitized production of singlet oxygen involves energy transfer from the excited triplet state of MB to molecular oxygen (Eq. (2))

$$^3MB^* + O_2\left(^3\sum_g^-\right) \rightarrow MB + O_2(^1\Delta_g). \quad (2)$$

2.3. Molecular Dynamics Simulations

The starting BSA, BLG and lysozyme structures were the X-ray diffraction structures 4F5S [23], 1BEB [24] and 1W6Z [25] respectively and were obtained from the RCSB Protein Data Bank. Each protein was

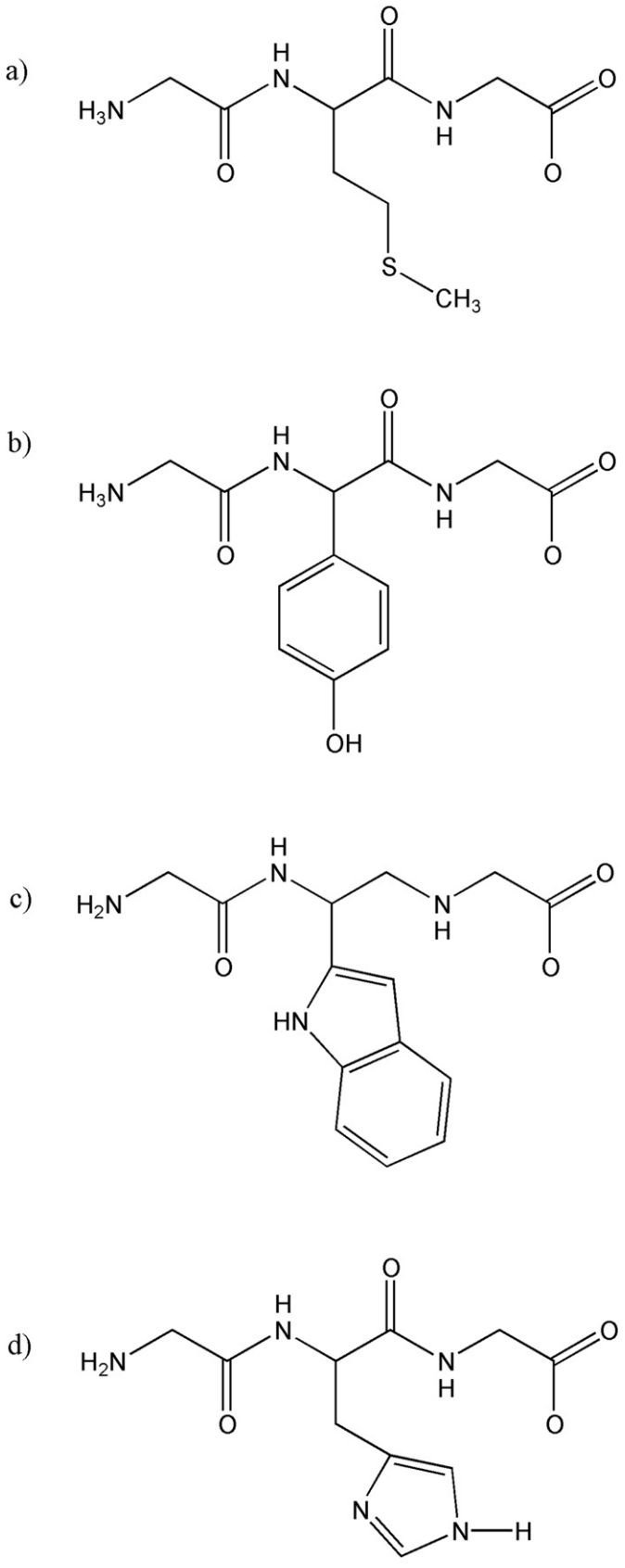

Fig. 1. Model molecules used in the present study: a) GlyMetGly b) GlyTyrGly c) GlyTrpGly d) GlyHisGly.