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Charge transfer between tryptophan and tyrosine in casein: a pulse radiolysis study

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Abstract

Charge transfer from tyrosine to tryptophan radicals in bovine milk casein, as observed using pulse radiolysis technique, is reported. The reactions of casein with hydroxyl, azide, $Br_2^{\bullet -}$ and $CCl_3O_2^{\bullet}$ radicals have also been studied. Radical transformation was found to take place at a rate of 1.5×10^4 s^{-1}. The effect of pH, oxidising radical and the proximity of tyrosine and tryptophan on this radical transformation, as well as repair of the casein radical by ascorbate, have also been studied. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Bovine milk casein; Radical transformation; Repair; Pulse radiolysis

1. Introduction

The pulse radiolysis technique has previously been used to demonstrate radical transformation involving charge transfer in simple model peptides [1–3] and proteins [3–6]. Many protein reactions involve charge transfer, including those initiated by light or ionising radiation. One possibility is that proteins might act as semiconductors when electrons are removed by the action of electron acceptors. Efficient intramolecular charge transfer was found to occur between tryptophan and tyrosine after one-electron abstraction from tryptophan units of small peptides and proteins in aqueous solution. Selective one-electron abstraction can be achieved by reaction with the azide radical, which has no absorption beyond 300 nm, as well as by the $Br_2^{\bullet -}$ radical. This charge transfer is affected by the distance, the difference in redox potential between the donor and the acceptor, the solvent structural reorganisation accompanying the transfer, etc. Charge transfer from tyrosine to tryptophan radical also depends on the three-dimensional structure of the protein molecule. In erabutoxin-b, the tryptophan radical did not transform into the tyrosine radical until the –S–S– bonds were broken, despite the close proximity of tryptophan and tyrosine [3]. This showed that peptide bonds do not provide a channel for electron transfer and that direct contact between the two reaction centres is a prerequisite. Casein (α_{s1}), a major (36.4 mol%) component of casein, is a perfect case for this prerequisite, which has two tryptophan and eight tyrosine amino acid moieties with adjacent tryp-

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tophan (164) and tyrosine (165) in its linear polypeptide chain [7].

2. Experimental

The pulse radiolysis system using 7-MeV electrons has been previously described [8]. Dosimetry was carried out using an air-saturated aqueous solution containing 5×10^{-2} mol dm^{-3} KSCN ($G\varepsilon=2.6\times10^{-4}$ m^2 J^{-1} at 475 nm [9]). The kinetic spectrophotometric detection system covered the wavelength range from 250 to 800 nm. The optical path length of the cell was 1.0 cm. The width of the electron pulse was 50 ns and the dose was 14 Gy. Casein (95% purity) from bovine milk was from Sigma and was used as received. All other chemicals were of analytical reagent grade. The pH values were obtained by adding 2×10^{-3} mol dm^{-3} phosphate buffer or NaOH. High-purity (>99.9%) N_2O from BOC India Pvt Ltd was used as required. The bimolecular rate constants were calculated by plotting the pseudo-first-order rate of formation of the solute radical or decay of the transient against the respective solute concentration. Uncertainty in the measurement of the bimolecular rate constant was ±10%.

3. Results and discussion

Casein reacted with the azide radical at pH 6.3 to yield transient absorption maxima at 410 and 510 nm at 15 μs after the electron pulse (Fig. 1). The rate constant for this reaction measured at 410 (first part, a fast step) and 510 nm is 5.5×10^8 and 3.1×10^9 dm^3 mol^{-1} s^{-1}, respectively. In addition to a rapid increase in absorption (first step) at 410 nm, a delayed increase (second step) in absorption has also been observed. There was simultaneous decay of absorption at 510 nm (tryptophan radical) and increase in absorption at 410 nm (tyrosine radical) (inset of Fig. 1). This radical transformation, which is due to charge transfer, takes place at a rate of 1.5×10^4 s^{-1}, as measured by both the decay rate of absorption at 510 nm and the increase at 410 nm. This radical transformation rate agrees well with that reported for tryptophan–tyrosine dipeptide [10]. The rate constants for the reaction of casein with the azide

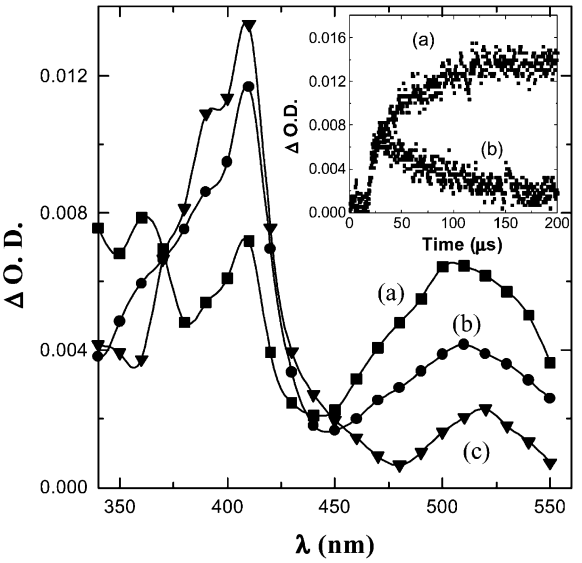

Fig. 1. Transient absorption spectrum obtained from N_2O-saturated aqueous solution containing casein (1×10^{-4} mol dm^{-3}), N_3^- (1×10^{-2} mol dm^{-3}) at pH 6.3 at: (a) 15; (b) 60; and (c) 180 μs after the electron pulse. Inset: kinetic traces at pH 6.3 at (a) 410 and (b) 510 nm for the above solution. Dose = 14 Gy.

radical measured both at 410 and 510 nm, at pH 10.5, have been found to be 1.7×10^{10} dm^3 mol^{-1} s^{-1}. At pH 10.5, no significant radical transformation was evident. Transient absorption at 510 nm was found to decay at a rate of $\sim1.2\times10^4$ s^{-1}, with first-order data fitting at pH 10.5 also. The casein molecule precipitates at pH <5.8, and at pH >11 it is not of much relevance to study such reactions.

Similar radical transformation was found to take place when casein reacted with a stronger one-electron oxidant, the $Br_2^{\bullet-}$ radical, at pH 6.8 (Fig. 2), also at the same rate of 1.5×10^4 s^{-1}. The bimolecular reaction rate constant measured by observing the decay of the $Br_2^{\bullet-}$ radical at 360 nm is 1.5×10^9 dm^3 mol^{-1} s^{-1}. The trichloromethyl peroxyl radical ($CCl_3O_2^{\bullet}$), which is known to mainly react by addition and electron transfer, reacted with casein to show similar radical transformation at the same rate (figure not shown). In this case, decay and growth of transient absorption at 460 and 410 nm, respectively, were found to take place simultaneously. The 460-nm transient

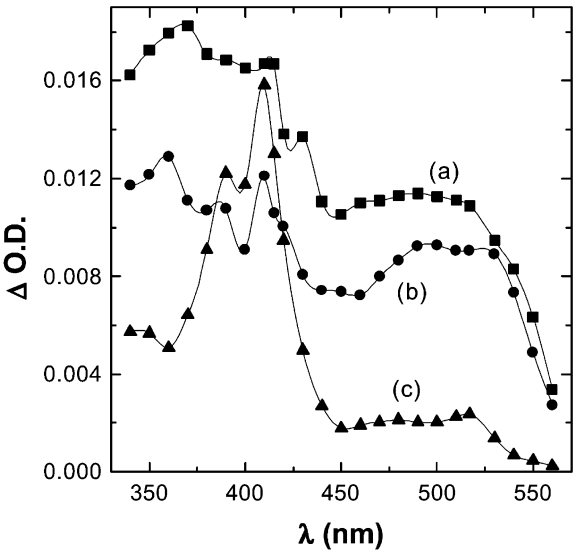

Fig. 2. Transient absorption spectrum obtained from N_2O-saturated aqueous solution containing casein (1×10^{-4} mol dm^{-3}) and KBr (1×10^{-1} mol dm^{-3}) at pH 6.8 at: (a) 10; (b) 18; and (c) 210 μs after the electron pulse. Dose = 14 Gy.

absorption observed in this case is due to the tryptophan radical, which has also been observed by us in the reaction of tryptophan and bovine serum albumin with the $CCl_3O_2^{\bullet}$ radical in a glycerol, propan-2-ol and water mixture. The transient absorption produced by $CCl_3O_2^{\bullet}$ at 460 nm is under further investigation, but is due to the tryptophan radical only, as has been observed with tryptophan alone. The reactions of casein with the $^{\bullet}OH$ radical do not show such radical transformation, since it is known to react by addition, electron transfer, etc., and not by selective one-electron oxidation. Casein reacted with $^{\bullet}OH$ and $CCl_3O_2^{\bullet}$ radicals with a bimolecular rate constant of 2.6×10^{10} and 5.1×10^{8} dm^3 mol^{-1} s^{-1}, respectively, measured at pH 6.8 and 410 nm. In the reaction of casein with the $^{\bullet}OH$ radical, the transient absorption corresponding to the tryptophan radical becomes a clear absorption maximum at 40 μs after the pulse, whereas for the tyrosine radical, it does not change. The rate constants for the reactions studied are given in Table 1. This study suggests that reaction with one-electron oxidants only shows the radical transformation as

Table 1
Rate constants for the reaction of casein with oxidising radicals

Radical	pH	λ (nm)	k (dm^3 mol^{-1} s^{-1})
$N_3^{\bullet}$	6.3	410	5.5×10^{8}
	6.3	510	3.1×10^{9}
	10.5	410, 510	1.7×10^{10}
$Br_2^{\bullet-}$	6.8	360	1.5×10^{9}
$CCl_3O_2^{\bullet}$	6.8	410	5.1×10^{8}
$^{\bullet}OH$	6.8	410	2.6×10^{10}

observed in earlier reports with model peptides and proteins.

The reaction of casein (1×10^{-4} mol dm^{-3}) with the azide radical in the presence of ascorbate (5×10^{-5} mol dm^{-3}) at pH 6.8 shows both the tryptophan → tyrosine and tyrosine → ascorbate radical transformations (Fig. 3). The tyrosine radical of casein has been found to react with ascorbate at a rate of 5.7×10^{8} dm^3 mol^{-1} s^{-1}, causing the transient absorption for the tyrosine radical (at 410 nm) to decrease and that for the ascorbate radical (at 360 nm) to increase (Fig. 3 and inset). It should be noted that the transient absorption at

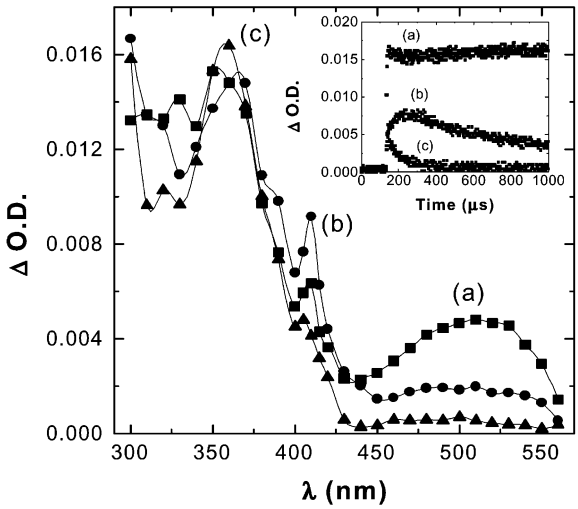

Fig. 3. Transient absorption spectrum obtained from N_2O-saturated aqueous solution containing casein (1×10^{-4} mol dm^{-3}), N_3^- (1×10^{-2} mol dm^{-3}) and ascorbate (5×10^{-5} mol dm^{-3}) at pH 6.8 at: (a) 10; (b) 60; and (c) 775 μs after the electron pulse. Inset: kinetic traces for the above solution at pH 6.8 at: (a) 360; (b) 410; and (c) 510 nm. Dose = 14 Gy.

360 nm decreases in the absence of ascorbate (Fig. 1). In this case, the concentrations of casein and ascorbate used were selected to simultaneously observe both the radical transformation and repair of the tyrosine radical by ascorbate.

α_{s1}-Casein is a linear polypeptide having two tryptophan and eight tyrosine amino acid moieties. Its three-dimensional structure is not well-defined, making it difficult to comment on distance-dependent charge transfer. However, the adjacent tryptophan (164) and tyrosine (165) residues in its linear polypeptide chain seem to favour charge transfer from tyrosine to tryptophan. In aqueous solutions, tyrosine and not tryptophan is supposed to be at the molecule–water interface because of its nature. However, at neutral bulk pH, from a thermodynamic point of view, tryptophan should be oxidised first, with a subsequent charge transfer from the tyrosine to the tryptophan radical. Furthermore, the pH inside and over macromolecules, such as proteins, may vary from the bulk, resulting in altogether different reactions. For example, the small pockets within and over lysozyme (a protein) are known to have pH values far different from the bulk. Even the folding of the linear polypeptide chain cannot be ruled out as reducing the distance between the other tryptophan (199) and other tyrosine residues.

In the present study, if the reaction of the azide radical with tryptophan and tyrosine residues is considered for simplification, then at pH 6.3, nearly $\sim$50% of the azide radicals directly react with each of them. This is based on the comparative value of the rate constant of the reaction of tryptophan/tyrosine with the azide radical, multiplied by the number of tryptophan/tyrosine residues in the molecule. Although there are different reports on the reduction potential of the tryptophan and tyrosine radicals, the reaction is thermodynamically favoured ($\Delta G^{o} = -8$ kJ mol^{-1}) in the forward direction at neutral pH [11,12]. The rate constant for the formation of tryptophan and tyrosine radicals at pH 10.5 is the same. Since casein has a higher number of tyrosine (eight) than tryptophan (two) residues, the majority (80%) of the azide radicals directly react with tyrosine, and a two-step reaction for the formation of the tyrosine radical has not been observed. The reaction of casein with $CCl_3O_2^{\bullet}$ suggests that even for the radicals that do not form adducts as final products, similar radical transformation takes place. The transformation of transient absorption for reactions of casein with $N_3^{\bullet}$, $Br_2^{\bullet -}$ and $CCl_3O_2^{\bullet}$ radicals and at the same rate clearly shows that this is due to charge transfer from the tyrosine to the tryptophan radical. This study further consolidates the hypothesis that radical transformation from tryptophan to tyrosine in a protein molecule depends on the difference in the redox potential of the donor and the acceptor, as well as on the proximity of the two. This charge transfer also depends on the pH of the solution, which decides the state of protonation, and thus the reactivity of tyrosine with oxidants. This further suggests that even in a polypeptide chain, such as casein, tyrosine amino acid retains its chemical characteristics.

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