Trp' → TyrO' RADICAL TRANSFORMATION IN HEN-EGG WHITE LYSOZYME

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Efficient intramolecular radical transformation Trp' → TyrO', involving long range electron transfer (LRET), between the phenol side chain of Tyr and the Trp' indolyl radical, has been observed in a number of proteins [1], including hen egg-white lysozyme (HEWL) [1-3] and in model systems made of a single Trp/Tyr pair separated by a peptide bridge [4-9]. There is also accumulating evidence that tyrosine and tryptophan radicals are involved in electron transfer associated with physiological redox reactions. So that further studies on LRET in HEWL, as model protein system for Trp' → TyrO' transformation, are worth of continuing. Our preliminary pulse radiolysis study on temperature dependence of LRET in HEWL [2] showed a close similarity between the Arrhenius plots for the kinetics of this process and the specific enzymatic reaction of HEWL, suggesting that similar thermal-ly induced conformational fluctuations are involved in the activation of the two processes in lysozyme.

So far it proved impossible, however, to identify which of the potential Trp'/Tyr redox pairs, formed by six tryptophan and three tyrosine residues present in HEWL, and which of the associated molecular pathways were actually involved in the observed LRET.

To identify which of the potential Trp'/Tyr redox pairs are actually involved in the N3-induced Trp' → TyrO' transformation in HEWL, we performed further pulse radiolysis studies of this reaction in: (i) the native enzyme, (ii) the complex of HEWL with an oligosaccharide inhibitor, triacetylchitotriose, and (iii) HEWL selectively oxidized by ozone to the NFKyn62 derivative (Fig.1). Both temperature and pH perturbations of the HEWL structure were employed [10]. The results obtained are interpreted in terms of the most probable LRET pathways between various Trp'/Tyr redox pairs in HEWL, calculated with help of the PATHWAYS model [11].

Intramolecular long range electron transfer (LRET) in hen egg-white lysozyme (HEWL), accompanying Trp' → TyrO' radical transformation, was investigated in aqueous solution by pulse radiolysis as a function of pH (5.2-7.4) and temperature (283-328 K). The reaction was induced by high selective oxidation of Trp with N3 radicals under

Fig.1. Spectrophotometric control of oxidation of Trp62 indole side chain to N'-formylkynurenine in HEWL, by ozone; the difference between the spectra of native HEWL and NFKyn62-HEWL corresponds to 1:1 conversion of 1 mole of Trp into NFKyn; in the inset a part of the absorption spectrum due to O-formylaminobenzoate group of NFKyn.

Fig.2. Dependence of the rate constant for reaction ket on pH in native HEWL, and in H-Trp-(Pro)5-Tyr-OH peptide (inset: ket vs. pH; Bobrowski et al., unpublished results).

Fig.3. Arrhenius plots for the temperature dependence of the rate constant, ket, for native HEWL at pH values indicated: Ea - corresponding energies of activation, derived from the slopes of the plots.

The reaction is induced by a high concentration of the reactants but with a high HEWL/N3 molar ratio so that more than 99 per cent of the oxidized protein molecules contained only a single tryptophyl radical. Synchronous decay of Trp' and build up of TyrO' conformed satisfactorily to first-order kinetics indicating that LRET involved either one or more Trp'/Tyr redox pairs characterized by similar rate constants. The rate constant of LRET, ket, increased monotonically
with decreasing pH (Fig.2) in the manner characterized by: (i) in the pH range 7.4-5.2 the plot of $k_{et}$ vs. pH was sigmoidal in shape reflecting protonation of Glu35 ($pK_a \approx 6$) and indicating involvement of a conformational control of the kinetics of LRET, below pH 5.2 a sharp increase in $k_{et}$ was observed due to the protonation of Trp$^\cdot$ to form TrpH$^++$ which is known to oxidize tyrosine faster. Arrhenius plots of the temperature dependence of $k_{et}$ showed that activation energy of LRET varies both with the state of protonation of the enzyme and the temperature (Fig.3). The activation energies are in the range 7.6-56.0 kJ mol$^{-1}$ and are similar to those for activation of amide hydrogen exchange in native HEWL below its denaturation temperature. Selective oxidation by ozone of the Trp62 indole side chain in HEWL to N'-formyl-kynurenine caused a large drop in the initial of Trp$^\cdot$ radicals, G(Trp)$^\cdot$. This was accompanied by a relatively small decrease in $k_{et}$ but selective oxidation by ozone had a profound change in its temperature dependence (Fig.4). Taken together these observations indicate that of the six tryptophans present in HEWL Trp62 contributes about 50% to the yield of the observed LRET. In the enzyme-inhibitor complex, HEWL(GlcNAc)$^3$, where Trp62 and Trp63 are completely shielded from the solvent by the bound triacetylchitriose, G(Trp)$^\cdot$, was lower than in NFKyn62-HEWL, and both the kinetic and energetic characteristics of LRET, observed only at pH 5.2 (Fig.4), were again somewhat different than in HEWL alone. Considering known solvent accessibilities of tryptophans in the complex, the observed LRET process in HEWL(GlcNAc)$^3$ was assigned to Trp$^\cdot$123.

Theoretical evaluation of the electronic coupling for the dominant LRET pathway between all the potential Trp /Tyr redox couples in HEWL, with help of the PATHWAYS model, allowed Trp62/Tyr53, Trp63/Tyr53 and Trp123/Tyr23 to be identified as the pairs involved in the electron transfer observed experimentally [10].

References

SENSITIZED PHOTOOXIDATION OF METHIONINE-CONTAINING PEPTIDES IN AQUEOUS SOLUTION

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Carbonyl triplets can be potent oxidizing agents in biological materials. These triplets have been produced in biological systems, by both enzymatic and non-enzymatic methods [1]. Recently, the benzophe-