Vol. 96, No. 1, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 16, 1980 Pages 408-414

DIRECT DEMONSTRATION OF ELECTRON TRANSFER BETWEEN TRYPTOPHAN AND TYROSINE IN PROTEINS

Walter A. PRÜTZ,* John BUTLER,† Edward J. LAND† and A. John SWALLOW†

* Universität Freiburg, Institut für Biophysik und Strahlenbiologie, Albertstr. 23, D-7800, Freiburg (Germany); † Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, M20 9BX (U.K.)

Received August 4,1980

SUMMARY: With several proteins it has been shown that electrons can be transferred intramolecularly from tyrosine to electron-deficient tryptophan units. Rates vary from ~ $10^2 s^{-1}$ (in lysozyme) to ~ $2x10^4$ s^{-1} (in trypsin). For β-lactoglobulin the activation energy is 45kJ mol^{-1}. This is incompatible with charge conduction along the polypeptide chain and rules out any mechanism involving temperature-labile hydrogen bonds as the main pathway. It seems likely that the electron transfer proceeds directly between the aromatic groups, while they are maintained at a distance from each other.

Electron transfer within proteins is of general importance in biological oxidation-reduction systems, as well as being of special interest for radiobiology and photobiology. It has been thought possible that aromatic units such as TrpH and TyrOH could be implicated in such processes (1) although there is also evidence to the contrary (2). Intramolecular electron transfer between electron-rich or -deficient TrpH and TyrOH and the other amino acid is not fast in free aqueous solutions (3), but intramolecular electron transfer from TyrOH to electron deficient TrpH ($TrpH^{\cdot +}$ and $Trp^{\cdot}$) has already been demonstrated in peptides (4, 5, 6) eg.:

Abbreviations: TrpH: tryptophan; TyrOH: tyrosine; β-La (A+B): β-lactoglobulins A+B; SDS: sodium dodecyl sulphate

0006-291X/80/170408-07$01.00/0

$$Trp^{\cdot}\text{-}(Gly)_n - TyrOH \longrightarrow TrpH\text{-}(Gly)_n - TyrO^{\cdot}$$

-where n = 0-3.We have now generated electron deficient TrpH units in several proteins by subjecting them to the action of $N_3^{\cdot}$ radicals generated from pulse-radiolytically produced OH radicals (7) and have obtained direct evidence of electron transfer from TyrOH units to the electron-deficient TrpH.

MATERIALS AND METHODS

α-Chymotrypsinogen A, concanavalin A, α-lactalbumin and β-La were obtained from Sigma, pepsin and trypsin from Serva and lysozyme from Boehringer. Solutions contained protein at concentrations of 0.7-8.0 mg/ml and NaN_3 at 20-50 mmol dm^{-3}. They were saturated with N_2O (room temperature) and were unbuffered except for β-La (A+B) which was also investigated in buffers (Fig.1). The Paterson Laboratories pulse radiolysis facility (8) was used to give doses such that the concentration of radicals introduced into the system was < 10% of that of the TrpH units present.

RESULTS AND DISCUSSION

Fig.1 shows typical results, obtained by allowing $N_3^{\cdot}$ to react with β-La (A+B), at two different pH values. It can be seen that by 20μs the $N_3^{\cdot}$ radicals had reacted to form a species with a broad absorption peaking around 510 nm, typical of the deprotonated electron-deficient TrpH (indolyl) radical, $Trp^{\cdot}$ (9, 10). Subsequently the absorption spectrum changed (over a few hundred microseconds) revealing a new product with absorption peaks close to 390 and 410 nm, characteristic of the deprotonated electron-deficient TyrOH (phenoxyl) radical, $TyrO^{\cdot}$ (11). The transformation followed first order kinetics at every wavelength examined, the rate being independent of the protein concentration (Table 1) and the same at pH 6.1 as at pH 7.9 (Fig.1 insert). The possibility of involvement of cystinyl radical cations exhibiting strong absorption around 400 nm (12) was ruled out by separate experiments which showed that $N_3^{\cdot}$ was very inefficient at generating such species in reaction with cystine-bis-glycine and cystine-bis-

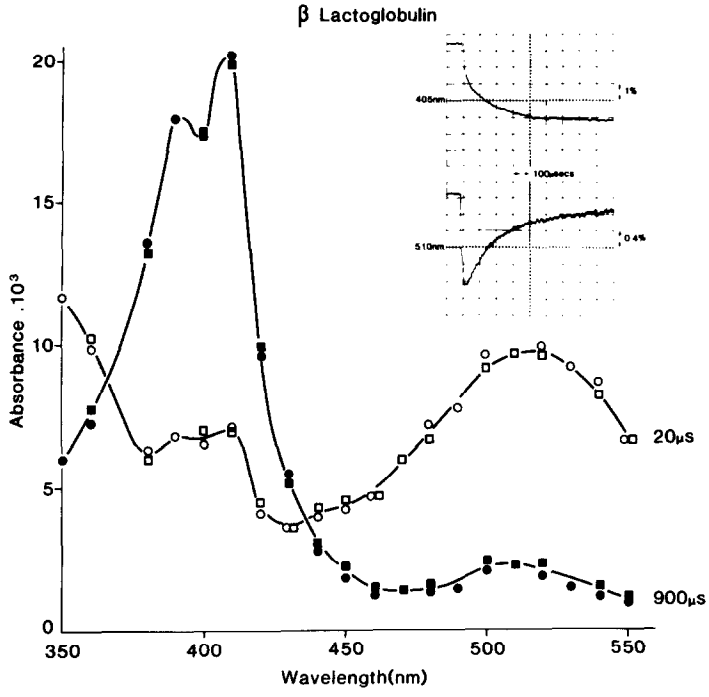

Figure 1. Transient absorptions in N_2O-saturated aqueous solution of 3.7 mg/ml β-La (A+B) + 50 mmol $dm^{-3}NaN_3$. The circles refer to pH 6.1 (10^{-2} mol dm^{-3} phosphate buffer) and the squares to pH 7.9 (10^{-2} mol dm^{-3} phosphate buffer). Inserted are oscilloscope traces of the transmission changes at 405 nm (top) and 510 nm (bottom) for the solution at pH 6.1: traces obtained at pH 7.9 were identical to those at pH 6.1.

tryptophan. Assignment of the absorptions at 510 nm and 405 nm to indolyl and phenoxyl radicals respectively, together with the initial yield of $N_3^{\cdot}$, enables values to be given for the percentage of $N_3^{\cdot}$ converted to the organic radicals. For the experiments of Fig.1, the maximum observable yield of indolyl radicals corresponded to about 50% of the $N_3^{\cdot}$, while the yield of phenoxyl radicals present before the slow reaction corresponded to about 20% of the $N_3^{\cdot}$. By 900μs the indolyl radicals corresponded to only 5% of the $N_3^{\cdot}$, while phenoxyl radicals corresponded to 70%. The slow loss of 45% indolyl while 50% phenoxyl was formed at the same rate shows that electron transfer between oxidized TrpH units and native TyrOH units, as previously observed in peptides, also proceeds efficiently in β-La.

TABLE I

RATE CONSTANTS FOR INTRAMOLECULAR CONVERSION OF INDOLYL INTO PHENOXYL RADICALS

Protein	Number of TrpH	Number of TyrOH	Concentration mg/ml	pH	Method**	Rate Constant s^{-1}
α-chymotrypsinogen A	8	4	4.0	6.1	D and B	$\sim 2\ 10^3$
					D only	$\sim 6\ 10^2$
Concanavalin A	4	7	2.6	6.7	D and B	$4.5\ 10^3$
α-Lactalbumin	4	4	2.8	7.4	D and B	$9.7\ 10^3$
					D only	$\sim 6\ 10^2$
β-La A	2	4	3.7	6.2	D and B	$5.7\ 10^3$
β-La(A+B)	2	4	0.7-8.0	6.1-7.9	D and B	$5.6\ 10^3$
β-La(A+B) + 0.5 mol dm^{-3} $NaClO_4$	2	4	4.0	6.1	D and B	$7.5\ 10^3$
β-La(A+B) + 30 mmol dm^{-3} SDS	2	4	4.0	6.8	D and B	$2.8\ 10^3$
β-La(A+B) + NaOH	2	4	4.0	11.2	D and B	$\sim 2\ 10^4$
Lysozyme	6	3	0.7	7.0	B only	10^2
Pepsin	5	17	7.2	5.9	D and B	$1.4\ 10^4$
					D only	$\sim 3\ 10^3$
Trypsin	4	10	4.8	5.6	D and B	$\sim 2\ 10^4$
					D only	$\sim 3\ 10^3$

** D = 510 nm decay kinetics, B = 405 nm build-up kinetics; D and B results were consistent in most cases within an accuracy of ∿ 15% (the mean is given). Independence of dose was verified for α-chymotrypsinogen, β-La(A+B) and pepsin. Rate constants refer to room temperature conditions.

Results like those with β-La have also been obtained with a number of other proteins. Rate constants for the electron transfers are collected in Table 1. Less pronounced transfers have been seen with yeast alcohol dehydrogenase (7), bovine γ-globulins and papain. Since the proteins investigated contain a number of TrpH and TyrOH residues (Table I) there is a possibility of several simultaneous electron transfer reactions. The disappearance of the 510 nm absorption in some of the proteins provided indications of more than one rate, and these additional rates are also included in the Table. However, in some of these cases the simultaneous appearance of the 405 nm absorption was masked by the final disappearance of phenoxyl radicals at rates which depended on the concentration of radicals introduced into the system, so that indolyl radicals could have been disappearing without reaction with TyrOH. The very slow rates (e.g. for lysozyme) are considered less certain also in view of possible slow intermolecular radical transformations. It is tempting to attribute the faster transfers to reactions between nearest neighbour groups in the polypeptide chain and the slower rates, if real, to pairs with a greater separation - thus the fast transfer, e.g. in trypsin ($2.10^4 s^{-1}$) may involve the residues TyrOH-218 and TrpH-221 and the slow transfer ($3.10^3 s^{-1}$) the residues TrpH-40 and TyrOH-48. In the TrpH-$(Gly)_n$-TyrOH peptides previously investigated (5) the transfer rates in fact decrease when going from n=0 to n = 2 but increase again for n = 3. Since proteins are more rigid than these peptides previously investigated (4, 5, 6) the rates should decrease more continuously with the number of residues between the reactants. However, the globular structure of the protein could promote other TrpH/TyrOH interactions. β-La itself, in both variants, has the adjacent groups TrpH-19 and TyrOH-20 (13). It might be expected that this would lead to an observable transfer in the time-

scale of 10μs, similar to that in TrpH-TyrOH ($k = 7.3 \cdot 10^4 s^{-1}$) (5). However even at 8mg/ml no reaction in this time scale could be resolved. Since $N_3^{\cdot}$ does not necessarily attack all the TrpH residues equally, this implies that $N_3^{\cdot}$ attack at TrpH-19 is minor, so that the observed electron transfer must be initiated by electron deficient TrpH-61. No decision can be made as to which of the TyrOH residues in β-La (20, 42, 99 or 102) is the electron donor, since the tertiary structure of β-La is not sufficiently resolved to locate the aromatic amino acids (13).

The effects of $NaClO_4$ and SDS on the transfer rate in β-La (Table I) are attributed to conformational changes modifying the distances between the reacting groups, $NaClO_4$ promoting contraction or clustering and SDS unfolding. However the characteristic conformational change of β-La at pH 7 (known as the Tanford transition,(13)) produces no change in transfer rate as shown by the fact that the rate is the same at pH 6.1 as at pH 7.9. A very fast conversion of indolyl into phenoxyl radicals in β-La could be demonstrated at pH 11.2 (Table I) even though deprotonation of the phenol group (pK_a = 10.1) promotes $N_3^{\cdot}$ attack at TyrOH ($k(N_3^{\cdot} + TrpH)/(N_3^{\cdot} + TyrO^-) \sim 1.2$) (7). As in the case of peptides (5) we conclude that the increase in rate is indeed due to TyrOH deprotonation even though conformational changes in the protein may contribute. This provides further evidence that the observed radical transformations proceed by electron transfer rather than H-atom transfer.

In the range 7°C to 64°C the electron transfer between TyrOH and $Trp^{\cdot}$ in β-La(A+B) was found to exhibit a reversible increase with temperature. The temperature dependence was compatible with an Arrhenius-type activation of the transfer process ($\ln k \propto -E/\underline{k}T$, where $\underline{k}$ = gas constant), with an apparent activation energy of E = 45kJ mol^{-1}. As in the case of peptides (5) the thermal activation

is not compatible with a mechanism involving (temperature-labile) hydrogen bonds as the electron migration path, and the activation energy is lower than would have been expected for charge conduction through the conduction band of the polypeptide chain. The observed activation is probably due to thermal broadening of the distance distribution for the reacting groups. That more activation energy is required with β-La than with TrpH-TyrOH (21 kJ mol^{-1} (5)) appears to reflect the higher rigidity of the protein, the structure of which is stabilized by disulfide bonds (two) as well as by non-covalent bonds.

ACKNOWLEDGEMENTS

This work was supported by a grant (Pr 178/1) from the Deutsche Forschungsgemeinschaft (Germany) and by grants from the Cancer Research Campaign and the Medical Research Council (U.K.).

REFERENCES

1. Winfield, M.E. (1965) J.Mol.Biol. 12, 600-611
2. Burns, P.S., Harrod, J.F., Williams, R.J.P. and Wright, P.E.(1976) Biochim.Biophys.Acta 428. 261-268
3. Swallow, A.J. (1977) Disc.Faraday Soc. 63, 289-290.
4. Prütz, W.A. and Land, E.J. (1979) Int.J.Radiat.Biol. 36, 513-520.
5. Prütz, W.A., Land, E.J. and Sloper, R.W. J.Chem.Soc.Faraday Trans. I (in the press).
6. Land, E.J. and Sloper, R.W. Photochem.Photobiol. (in the press).
7. Land, E.J. and Prütz, W.A. (1979) Int.J.Radiat.Biol. 36, 75-83
8. Keene, J.P. (1964) J.Scient.Instrum. 41, 493-496.
9. Santus, R. and Grossweiner, L.I. (1972) Photochem.Photobiol. 15, 101-105.
10. Posener, M.L., Adams, G.E., Wardman, P. and Cundall, R.B. (1976) J.Chem.Soc.Faraday Trans I 72, 2231-2239.
11. Land, E.J. and Porter, G. (1963) Trans.Faraday Soc. 59 2016-2026.
12. (a) Bonifačić, M.,Schäfer, K.,Möckel, H. and Asmus, K.-D, (1975) J.Phys.Chem. 79, 1496-1502; (b) Bonifačić, M. and Asmus, K.-D. (1976) J.Phys.Chem. 80, 2426-2430.
13. Green, D.W., Aschaffenburg, R., Camerman, A., Coppola, J.C., Dunnill, P., Simmons, R.M., Komorowski, E.S., Sawayer, L., Turner, E.M.C. and Woods, K.F. (1979) J.Mol.Biol. 131, 375-397