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Electrostatic Effect of Trypsin Binding on the Hydrogen Exchange Rate of Bovine Pancreatic Trypsin Inhibitor β-sheet NH's

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The changes of H–D exchange rates upon protein–protein interactions are generally interpreted as a result of the changes of the dynamic properties of the proteins. The effect of trypsin binding on the H–D exchange kinetics of some trypsin inhibitor amide H's was reported (Simon *et al.*, 1984). In this paper the electrostatic potential originating from the trypsin molecule is calculated at the positions of the studied amide H's in the trypsin-trypsin inhibitor complex. We conclude that the observed decrease of the exchange rates is mainly due to the electrostatic field of the trypsin molecule.

1. Introduction

BPTI‡ contains a group of peptide NH's which have extraordinarily slow exchange rates (Woodward & Hilton, 1980; Wagner & Wüthrich, 1982; Simon *et al.*, 1984). These NH's, sometimes referred to as β-core protons, are hydrogen-bonded in three strands of the β-sheet of the small, globular protein. In our recent paper (Simon *et al.*, 1984) we reported on the effect of trypsin binding on the hydrogen exchange kinetics of the BPTI β-core protons. We determined H–D exchange rates in free BPTI and in TIC‡ at pH 9–10 and in a temperature range of 25–40°C. Whereas none of the atoms of the studied residues are involved in intermolecular contacts of TIC (Janin & Chothia, 1976), trypsin binding has a highly localized effect. The exchange rate of Tyr-35 NH is lowered by more than three orders of magnitude in the complex while for Gln-31, Phe-33 and Phe-45 a considerably smaller effect was detected (Simon *et al.*, 1984).

It has been proposed (Salemme, 1982) that the conformational flexibility and hydrogen bond stability of the β-sheet structure in BPTI arise from the

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‡ Abbreviations used: BPTI, bovine pancreatic trypsin inhibitor; TIC, trypsin–BPTI complex.

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continuous interconversion between numerous flat and twisted β-sheet conformations via concerted motions that involve considerable excursions from the average crystal structure. The observation that H–D exchange rates of peptide NH's at the ends of the sheet (Gln-31 and Tyr-35) are much faster than those in the middle is cited as supporting evidence (Salemme, 1982).

A plausible explanation for the selective slow-down of exchange for Tyr-35 amide proton in the complex is that one or a few low-frequency collective modes are responsible for Tyr-35 NH exchange in free BPTI and these are eliminated in TIC (Simon *et al.*, 1984).

The dramatic decrease of exchange rate for Tyr-35 as compared to Gln-31, located at the opposite end of the β-core, the fact that after complexation this exchange becomes even slower than the corresponding process for amide protons in the middle of the β-sheet (Phe-33 and Phe-45) and the observation that trypsin binding results in no significant change in the activation enthalpies, suggest that there may be another explanation for the observed effect.

At pH values larger than 4 H–D exchange is a HO^--catalyzed process. Charged groups and dipoles may alter the local pH by expelling hydroxyl ions from the vicinity of the peptide NH's. This is in agreement with the observation that exchange rate constants for peptide protons in a randomly coiled polypeptide chain vary by more than an order of magnitude (Woodward & Rosenberg, 1970) due to the electrostatic effect of neighbouring side chains (Molday *et al.*, 1972) and the peptide NH exchange in highly charged poly-DL-lysine is subject to a large salt effect (Kim & Baldwin, 1982). A significant electrostatic effect on the exchange of β-sheet amide protons of BPTI originated from the other parts of the BPTI molecule was found in a study of the pH dependence of H–D exchange (Matthew & Richards, 1983).

2. Method

The electrostatic potential originating from the trypsin molecule at the NH proton sites of Gln-31, Phe-33, Tyr-35 and Phe-45 was calculated using a simple method (Náray-Szabó *et al.*, 1981; Ángyán & Náray-Szabó, 1983). It was supposed that the structure of TIC in solution is equivalent to that in the crystal therefore we used coordinates given by the Protein Data Bank (Huber & Deisenhofer, 1982). Electrostatic potentials were calculated by omitting the whole BPTI molecule from TIC and considering only the atoms of trypsin.

It is supposed that surface charged groups of trypsin are completely shielded by counter ions except Asp-189 which is located at the contact

surface of TIC and forms an ion pair with Lys-15 of BPTI. Asp-189 mimicks just the effect of a counter ion near Lys-15 of the free BPTI. Thus at pH 9-10 electrostatic potential changes in TIC relative to BPTI originate from the negatively charged buried Asp-102 side chain and dipoles near the contact surface of the trypsin molecule.

3. Results and Discussion

Results of our calculations are summarized in Table 1. It can be seen that the more negative the electrostatic potential the more pronounced is the effect of trypsin binding on rate decrease. Under experimental conditions, where the exchange data listed in Table 1 were obtained, H-D exchange is mediated by the penetration of catalytic hydroxyl ions (Woodward *et al.*, 1982). It is therefore very probable that the negative potential field expels the negative HO^- ions from the vicinity of exchangeable amide protons, i.e. the local pH decreases diminishing the exchange rate.

TABLE 1

Effective electrostatic potential of trypsin at BPTI β-sheet NH proton sites as compared to relative exchange rates in TIC. R is the distance of amide proton from the midpoint between $O^{\delta 1}$ and $O^{\delta 2}$ in Asp-102 (nm). V is the trypsin potential (kJ mol^{-1}) and ε is the dielectric paramater. $k^*_{rel} = k_{BPTI}\, k^{-1}_{TIC}$ (k^*_{rel} data taken from Simon et al., 1984)

Side chain of BPTI	R	$-V \cdot \varepsilon$		k^*_{rel}
		Asp-102	dipoles	
Gln-31	2.57	55	32	3 ± 1
Phe-33	2·02	69	35	15 ± 6
Phe-45	2·00	71	38	5 ± 3
Tyr-35	1·48	97	56	>1000

Table 1 shows that the main source of the electrostatic effect is the charged Asp-102 side chain. Putting $\varepsilon = 4$ (Bolis *et al.*, 1979) the difference between Asp-102 potentials for Gln-31 and Tyr-35 is 11 kJ mol^{-1} while for the remaining dipoles this difference is only 6 kJ mol^{-1}. The total effect is 17 kJ mol^{-1} in excellent agreement with the experimental energy change ($RT \ln k_{rel,Tyr\text{-}35} - RT \ln k_{rel,Gln\text{-}31} > 15$ kJ mol^{-1} at 25°C). Even considering that the value of ε is uncertain its magnitude may vary between 2 and 4

from author to author, it can be stated that the electrostatic field of trypsin molecule alone may result in the observed dramatic decrease of the exchange rate of NH of Tyr 35.

The primary importance of the electrostatic effect of Asp 102 of free trypsin have been suggested by a calculation similar to the one used in this work (Náray-Szabó, 1983). Later it was found experimentally that replacing Asp 102 by Asn leads to a decrease of the original trypsin enzyme activity by a factor of 20 (Craik *et al.*, 1985). We suggest that the estimations of this paper could be checked by measurements on TIC with a point mutant of trypsin where Asp-102 is changed to neutral Asn. If the electrostatic effect is as large as we suggest the decrease of exchange rate for Tyr-35 would be much less, only one order of magnitude.

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