HYDROGEN-DEUTERIUM EXCHANGE OF ANGIOTENSIN II IN TRIFLUOROETHANOL

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1. Introduction

(Asn[1], Val[5]) angiotensin is a linear octapeptide hormone which exhibits several important biological activities, especially a vasopressor action. Its structure in aqueous environment has been identified as a mixture of ordered and disordered peptide conformations [1,2]. However, circular dichroism, infrared, and Raman investigations indicate that in organic solvents such as trifluoroethanol, this hormone adopts the cross-β conformation which seems to be required for the hormone-receptor interaction [3].

Hydrogen isotope exchange measurements performed in aqueous solutions and recently in organic solvents [4,5] have often been used as a tool in studies of protein and peptide structures [6—9]. This method supplies pieces of information on the solvent accessibility to peptide bonds: the different exchange rates of labile hydrogens of a peptide reflect their different locations within the molecule. Therefore, hydrogen-deuterium exchange was used in this study to perfect our knowledge on the AN2 conformation in TFE: the exchange kinetics of peptide protons of the hormone dissolved in TFE were followed by infrared spectrometry [10,11] and their rate constants were compared with those of protons in a model amide compound, N-methylacetamide.

2. Materials and methods

2.1. Materials and instrumentation

AN2 was a gift from Dr Riniker (CIBA Geigy,

Abbreviations: AN2, (Asn[1], Val[5]) angiotensin II; TFE; deuterated trifluoroethanol.

Basel) and was used without further purification. TFE ($CF_3C^2H_2O^2H$, 98.4 isotopic purity) and ^2H_2O (99.7 isotopic purity) were obtained from the 'Commissariat à l'Energie Atomique'. N-methylacetamide was purchased from the Schuchardt Laboratories, Munich.

Infrared spectra were recorded on a Perkin Elmer 521 double beam spectrophotometer with an accuracy of approx. 1 cm^{-1}. Matched CaF$_2$ cells of 0.05 mm path length were thermostated by water jackets and the temperature inside the exchanging solution was directly measured with a chromel-alumel thermocouple. The reference cell contained pure TFE. Infrared spectra were digitalized as in previous infrared studies [12] and difference absorption spectra were computed on a PDP 12.

2.2. Exchange experiments

For each experiment, an aliquot of 300 μl of AN2 (5.5 10^{-2} M) in ^1H_2O was lyophilized in vacuo over P_2O_5 for 20 h at room temperature. The pH was measured on a solution in ^2H_2O of this lyophilized AN2 and was found equal to 6.7. The corresponding pH in TFE should be around 12 [13]; its exact value is not critical for this study since all experiments were carried out under the same conditions.

Prior to any exchange experiment, lyophilized AN2, TFE, syringes, and cells were kept at the selected temperature (11° or 20°C). The lyophilized peptide was dissolved in 300 μl of TFE and the solution was immediately transferred to the sample cell. The rate of the hydrogen-deuterium substitution was measured by following the absorbance decrease of the amide II band (1555 cm^{-1}) [11] between 3 min and 25 h; meanwhile the AN2 concentration was checked with the amide I band (1635 cm^{-1}) [10].

Completely deuterated AN2 (i.e., AN2 with its 6 potentially exchangeable peptide hydrogens replaced by deuterium) was obtained after up to nine deuteration steps (dissolution in ^2H_2O, warming for 4 h at 50°C, and lyophilization). Spectra of this deuterated AN2 dissolved in TFE were used as references for the measurement of amide I and amide II absorbances (resp., $A_{amide\ I}$ and $A_{amide\ II}$).

The initial ratio $(A_{amide\ II}/A_{amide\ I})_{t=0}$ for a completely hydrogenated AN2 was determined by extrapolation to zero time of the ratio $(A_{amide\ II}/A_{amide\ I})_t$ measured during the first 10 min of exchange at 11°C. This method yields a value of 0.38, which is somewhat low compared to those usually observed for proteins [10,12,14] but comparable to those proposed by Abrash [15] for metmyoglobin (0.39) and by Ralston [16] for β-lactoglobulin (0.37).

Consequently, the number of unexchanged peptide hydrogens per mole of AN2, H_t, at any time t, was calculated according to the equation

$$H_t = 6\ (A_{amide\ II}/A_{amide\ I})_t/0.38$$

3. Results and descussion

Infrared spectra of AN2 in TFE solution (11°C) at two stages of exchange are shown in fig.1. The amide II band at 1555 cm^{-1} corresponds to a coupled CN stretching and NH deformation frequency; it decreases upon deuteration of the CO–NH groups while the 1437 cm^{-1} band increases by about the same quantity. The amide I band at 1637 cm^{-1} arises from a CO stretching vibration of the peptide bond; the S-shape of the difference spectrum between 1660 cm^{-1} and 1620 cm^{-1} indicates that there is no change in integrated band intensity during the exchange but a 2 cm^{-1} shift and a narrowing at the apex. The position of the amide I band close to 1637 cm^{-1} and its weak, but reproducible shoulder around 1685 cm^{-1}, appear to be consistent with a significant contribution from a β conformation [17–20]. The 1515 cm^{-1} peak is due to tyrosine and its frequency remains constant with time. These observations are very comparable to those related by Fermandjian et al. [2] about infrared spectra of solid AN2 obtained from TFE solutions.
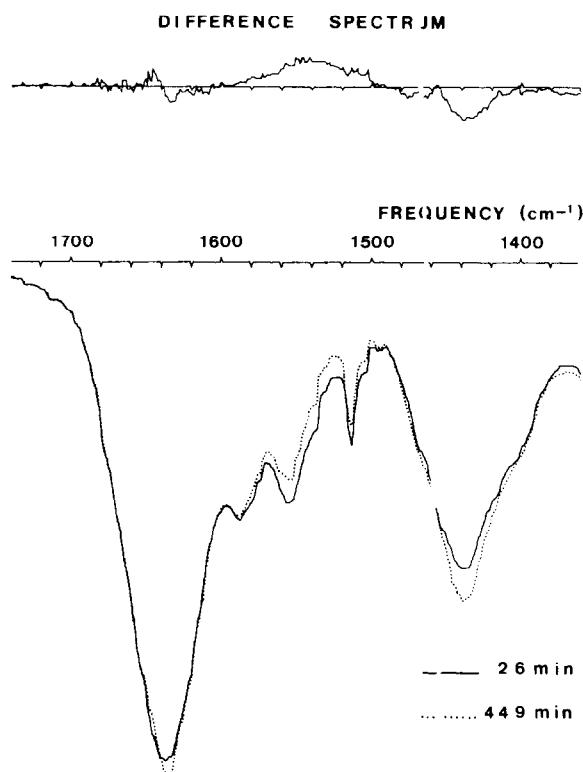
The exchange kinetics at 11° and 20°C of the pep-

Fig.1. Infrared spectra of AN2 (5.5 10^{-2} M) dissolved in deuterated TFE at 11°C: after 26 min of exchange (——) and after 449 min of exchange (. . . . .); the difference between these two spectra is presented on the upper graph.

tide hydrogens of AN2 are presented in fig.2. The rate of exchange vanishes after 150 min at 11°C and 30 min at 20°C. At 11°C, 0.5 peptide hydrogen exchanges rapidly before the first measurement (3 min); during the subsequent 25 h, 1.9 hydrogens exchange slowly while about 3.6 hydrogens remain unexchanged even at the end of this period. At 20°C, the corresponding values are 2, 1.2 and 2.8 hydrogens. These results are highly reproducible as it can be noticed in fig.2: the standard deviation is equal to 0.1 hydrogen. More precisely, graphical analyses of our data indicate two classes of exchanging atoms plus a core which is not exchanged after 25 h, even at 20°C. The rate constants and the half-times of these classes are given in table 1: when the temperature varies from 11° to 20°C, the rate constants are multiplied by 2 or 8, which should be compared with the tenfold increase
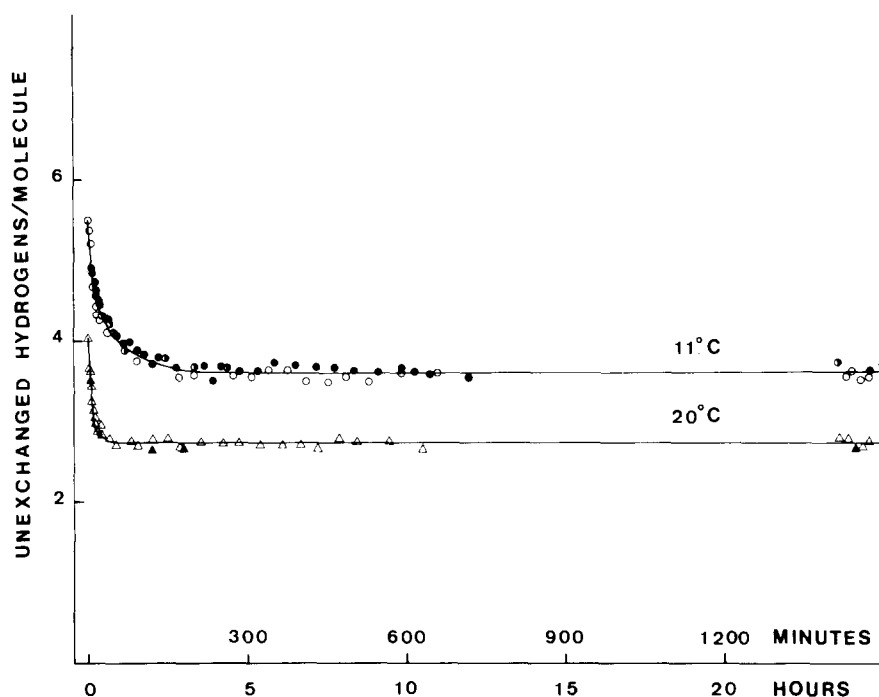
Fig.2. Exchange kinetics curves of AN2 at 11°C (○●◐) and 20°C (△▲).

per each 10°C change applying to freely exposed hydrogens of polypeptides dissolved in heavy water [21].

Printz et al. [8], have followed, with the tritium exchange technique, the slow exchange of two hydrogens in AN2 ($T_{1/2}$ = 300 min) at acid pH and in low concentrated aqueous solutions. We attempted to follow the hydrogen-deuterium kinetics of AN2 dissolved in ^2H_2O in the same conditions as in TFE: at 10°C and pH 6.7, 50% of the peptide hydrogens are already exchanged in the first minutes of the experiment, while the remaining 50% are not, even after 25

h. More precise results cannot be obtained due to the time resolution of the infrared technique. Moreover we have to use concentrations of the order of 0.05 M at which the hormone starts to aggregate in water but not in TFE, as shown by Rayleigh diffusion measurements [22]. Therefore infrared results obtained for ^2H_2O and TFE solutions cannot be directly compared since slowly exchanging hydrogens in aqueous solutions may correspond to inter-hormone as well as intra-hormone hydrogen bonds.

On the other hand, our kinetic results may be interpreted by comparison with data on model com-

Table 1
Class sizes, half-times and rate constants for the exchange of AN2 in TFE solutions

θ	C_I	$T_{1/2}$ min	k min^{-1} $\times 10^3$	C_{II}	$T_{1/2}$ min	k min^{-1} $\times 10^3$	C_{III}
11°C	1.4	5	138.6	1.0	43	16.1	3.6
20°C	1.2	3	231.0	2.0	5	138.6	2.8

pounds dissolved in TFE. N-methylacetamide is one of the simplest chemicals with an amide hydrogen presumably exposed to solvent. Its hydrogen—deuterium exchange was followed both in pure TFE and in TFE plus NaO^2H (with the concentration necessary to obtain a pH 6.7 solution in ^2H_2O). These exchange kinetics are of first order and their rate constants are equal respectively to 0.198 and 0.138 min^{-1}, which is of the same order of magnitude as those for fast exchanging hydrogens in AN2. Thus it can be assumed that, at $11°C$, at least one hydrogen of AN2 is freely accessible to TFE, another one is sterically hindered, while, in average, 3.6 hydrogens belong to hydrogen bond or are deeply buried in the molecule (these non-integer average values imply that the hormone structure corresponds to a family of similar conformations, even in a stabilizing solvent such as TFE). Other data from table 1 suggest that, at $20°C$, one hydrogen is freely exposed to TFE, while two are partially hindered and three belong to hydrogen bonds or are deeply buried in the hormone.

These results do agree with previous studies indicating a folding of the peptide chain in TFE according to a cross-β structure stabilized by several types of forces, including internal hydrogen bonds [1,2,23].

Acknowledgements

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