INFRA-RED INVESTIGATION OF THE STRUCTURAL TRANSITIONS OF TRYPSINOGEN, TRYPSIN, CHYMOTRYPSINOGEN AND CHYMOTRYPSIN

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1. Introduction

Timasheff et al. [1,2] recently studied the infra-red spectra in the region of the amide I band for a number of polypeptides and proteins in ^2H_2O solution. As a result, detailed assignments of amide I frequencies to various conformations (α-helix, β-structure, unordered conformation) have been established. We have used this technique to analyse the structural modifications undergone by trypsinogen, trypsin, chymotrypsinogen and chymotrypsin with changes of pH and temperature [3, 4].

2. Material and methods

Porcine trypsinogen was purified according to Charles et al. [5]. Trypsin was obtained from the activation of the zymogen. Bovine chymotrypsinogen A and α-chymotrypsin are Worthington products. Protein solutions were obtained by dissolving 6 mg of the lyophilized protein in 300 μl of ^2H_2O. p^{2}H was adjusted with ^{2}HCl or NaO^{2}H. Porcine trypsin was chosen because it is more stable than bovine trypsin [6]. Moreover, to eliminate any risk of autolysis, the experiments with this enzyme were carried out in solutions containing 10^{-2} M $CaCl_2$. Protein concentrations were calculated from the absorbance at 280 mμ. Infra-red spectra were recorded in a Perkin-Elmer model 225 spectrometer equipped with an air-dryer and a thermostatted cell-holder. Circular dichroism spectra were measured in a Fica spectropolarimeter-dichrograph.

3. Results and discussion

The results obtained for the four proteins at different pH and different temperatures are given in tables 1 and 2. Some typical spectra are presented in fig. 1. When the pH is decreased from about 8 to about 1.5–2 at 25°C, trypsinogens (Tg) and trypsins (Ti) undergo two successive transitions: I′ (pH 8) ⇌ II′ (pH 5) ⇌ III′ (pH < 2) for trypsinogen and I (pH 8) ⇌ II (pH 4) ⇌ III (pH < 2) for trypsin [3]. I′, II′, III′, I, II, III are conformational isomers. Under the same conditions chymotrypsinogens (ChTg) A and B [4] and chymotrypsin Aα (ChTi) [4, 7] apparently undergo only one transition: I′ ⇌ II′ for ChTg, I ⇌ II for ChTi. These last transitions as well as transitions II′ ⇌ III′ of Tg and II ⇌ III of Ti are controlled by the unmasking of carboxylate groups [3, 4]. At high temperatures all these species give rise to the thermally denatured forms D′ for the zymogens and D for the enzymes [3, 4].

The absorption maximum of the amide I band is at 1633 cm^{-1} for forms I′ and I of Tg and Ti, 1635 and 1634 cm^{-1} for forms I′ and I of ChTg and ChTi. A maximum near 1633 cm^{-1} is usually considered to indicate the presence of a β-conformation [8]. Consequently all these proteins probably have a fairly large amount of this type of ordered structure. The shift of the amide I band toward higher frequency (1637 cm^{-1}) after the unmasking of the buried carboxylates in forms III′ and III of Tg and Ti and II′ and II of ChTg and ChTi is consistent with a distortion of the β-structure as well as superposition of an absorption stemming from unordered regions at

Table 1
Characteristics of the Amide I band and extinction coefficients for ChTg A and αChTi

Protein	p^2H	Temp. (°C)	Isomeric form	ν_{max} (cm^{-1})	$[\epsilon]_{\nu_{max}}$	$[\epsilon]_{1645}$	$[\epsilon]_{1680}$	ν_s (cm^{-1})	$[\epsilon]_{\nu_s}$
ChTg A	1.40	25	II′	1637B	332		113	1665	209
	2.40	26	II′+I′	1636	386	356	121	1665	222
		50	40% (II′+I′) +60% D′	1640B	293	290	111	1665	201
	5.10	26	I′	1635	410	379	117	1665	228
	7.30	25	I′	1635	416	380	116	1665	227
αChTi	1.40	25	II	1638B	326	319	111		
	4.80	24	I	1635	399	369	131	1665	199
	6.80	25	I	1634	404	354	120	1665	226
	7.50	25	I	1634	410	360	132	1665	240
	12.80	27		1637B	348	336	109	1665	210

$[\epsilon]_\nu = \frac{\text{O.D.}}{1000} \times \frac{\text{MRW}}{l \times c}$. O.D. = optical density; MRW = mean residue weight; l = thickness of the cell in centimeters; c = concentration of the protein in g/ml. The precision of $[\epsilon]$ is 5%. ν_s is the frequency of the weak shoulder observed in the range 1660–1665 cm^{-1}. Typical values of $[\epsilon]$ at 1645 and 1680 cm^{-1} do not correspond to shoulder or maxima. The proportions of the different molecular forms were calculated from the results published in refs. [3, 4]. p^2H = pH + 0.4. B = Broad.

Table 2
Characteristics of the amide I band for Tg and Ti

Protein	p^2H	Temp. (°C)	Isomeric form	ν_{max} (cm^{-1})	$[\epsilon]_{\nu_{max}}$	$[\epsilon]_{1645}$	$[\epsilon]_{1680}$	ν_s (cm^{-1})	$[\epsilon]_{\nu_s}$
		25	III′	1637B	356	346	118	1665	224
Tg	1.6	40	20% III′ +80% D′	1640B	339	333			
		25	70% III′+30% II′	1637	380	369	144	1665	242
	2.30	50	25% III′+75% D′	1640B	324	324	124	1665	230
		70	D′	1445B	316	316	105	1670	212
	3.65	28	III′ + II′	1635	388	363	128	1660-1665	280 240
	5.50	26	II′	1633	402	368	128	1660-1665	282 246
	6.60	25	I′	1633	413	368	120	1660	274
	10.80	28		1639B	323	308		1660	241
Ti	1.95	27	III	1637B	356	339	117	1665	218
		50	50% III+50% D	1640B	332	330			
	3.45	26	II + III	1635	384	350	138	1665	239
	7.50	25	I	1633	403	350	111	1665	226

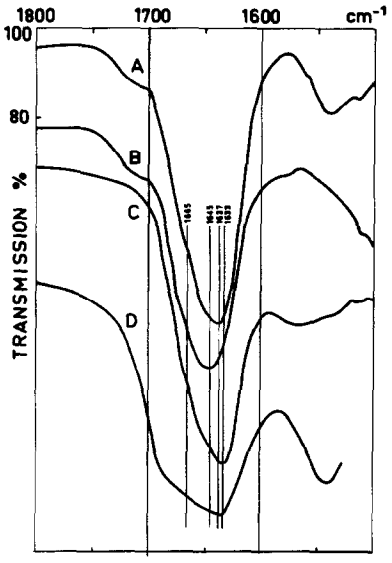

Fig. 1. Infra-red pattern of porcine trypsinogen and bovine chymotrypsinogen A. (A) Tg, pH 2.30, 25°C (B) Tg, pH 2.30, 70°C. (C) Tg, pH 6.60, 25°C. (D) ChTg A, nujol.

about 1645 cm^{-1}. It is quite clear with Tg (table 2 and fig. 1) that the β-structure present in form I′ is partially destroyed in form III′ and practically completely destroyed in D′, after the thermal denaturation of III′, to give rise to a completely unordered structure with a maximum at 1645 cm^{-1}. This result is in agreement with chemical evidence, which has shown for example that in bovine Tg, methionines 92 and 166 are masked in III′ and readily accessible to iodoacetamide and H_2O_2 in D′ [9].

Circular dichroism spectra of porcine Tg and ChTg A have been found to present a band at 217 mμ which is believed to characterize the antiparallel pleated sheet β-conformation [8]. For Tg the ellipticity of this band, $[\theta']$ varies from −2400 to −3000 deg.cm^2.$decimole^{-1}$ between pH 2 and 7.9. This result would also indicate a decrease of the percentage of β-structure when going from I′ to III′.

Measurements at alkaline pH with porcine Tg and ChTi (table 1 and 2) also show that the infra-red method can be used to follow isomerizations due to the unmasking of abnormal tyrosine residues.

No maximum can be detected at 1653 cm^{-1}, the position for α-helical regions [1]. This observation is not unexpected since Sigler et al. [10] have shown recently that only one α-helical region of more than one turn could be located in the structure of crystalline α-chymotrypsin. We consistently observed in all spectra a reproducible shoulder in the range 1660–1665 cm^{-1} which could be the result of overlapping of unresolved absorption bands arising from an α-helix band of low intensity superimposed on the weaker pleated sheet band (1680 cm^{-1} [1]) and a band due to unordered conformation (1645 cm^{-1}). This possible overlapp between different bands is most apparent with ChTgA in nujol (fig. 1). Circular dichroism spectra of porcine Tg and ChTgA also show a weak band at 222 mμ ($[\theta']$ = −3100 deg cm^2 $decimole^{-1}$) which is usually assigned to the α-helix [8];

The infra-red investigation, like ORD [11, 12], CD techniques and other physico-chemical methods [3, 4] which permit a conformational study of proteins in solution, show the striking similarity of the conformations of Tg and ChTg. In the last years, several authors [11–14] have tried, using ORD techniques, to find out the changes which occur in the ordered structure of ChTg in the course of its activation. More recently this problem was also studied by Willumsen using hydrogen-deuterium exchange [15]. Raval and Schellman [11] and Biltonen et al. [12] concluded that there was little or no change in the α-helix content. Their conclusion is consistent with the X-ray diffraction results [10]. The infra-red investigation described here shows that there is practically no difference (same values of ν_{max} and $[\epsilon]$) between corresponding forms of the precursors and the active enzymes (I and I′, II and II′, III and III′). The activation process will then probably involve very small changes not only of the α-helix content of the molecule but also of the β-structure. The activation of ChTg A (I′) into ChTi (I) is dependent upon the formation of an ion-pair between the carboxylate side-chain of aspartate 194 and the α-amino group of isoleucine 16 [10]. It is interesting to remark that the changes in the infra-red spectra which accompany this transformation are much smaller than those which are observed in transition II ⇌ I of ChTi which also correspond to the masking of one carboxylate group.

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