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Infrared Spectral Patterns of Thyroglobulin Bearing Thyroiditogenic Epitopes

Igor Cherepanov[1,4] · Alexandr Sidorov[1,2] · Liubov Beduleva[1,2] · Alexey Terentiev[1,2] · Daria Menshikova[1,3] · Tatyana Khramova[1,2] · Igor Menshikov[1,2] · Pavel Ivanov[1]

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Abstract
Thyroglobulin is a major autoantigen to which autoimmune response, destroying the thyroid gland in Hashimoto's thyroiditis, is directed. To detect a pathological autoimmune response to thyroglobulin, as well as the successful induction of experimental autoimmune thyroiditis, thyroglobulin carrying thyroiditogenic epitopes is necessary. It is not known which features of thyroglobulin structure determine the presence of thyroiditogenic epitopes and can serve as markers of their presence. We compared structure of thyroglobulin bearing thyroiditogenic epitopes (freshly isolated thyroglobulin) and thyroglobulin which had lost thyroiditogenic epitopes (lyophilized thyroglobulin). Fourier-transform infrared (FTIR) spectroscopy was used to elucidate the structure of thyroglobulin. The markers indicating the presence of thyroiditogenic epitopes on thyroglobulin are the vibrations of diiodotyrosine, monoiodotyrosine/diiodotyrosine relation in the range of 0.24–0.43 (95% confidence interval) and relatively high (> 32%) α-helix content. The loss of thyroiditogenic epitopes on thyroglobulin is associated with a weakening or complete disappearance of diiodotyrosine oscillations and a decrease in the proportion of α-helices in secondary structure. Thyroglobulin extracted with phenylmethylsulfonyl fluoride (PMSF) added is characterized by the same relatively high monoiodotyrosine/diiodotyrosine relation and low proportion of alpha helices as thyroglobulin without thyroiditogenic epitopes. Therefore, serine protease inhibitor PMSF is not suitable for extraction of native thyroglobulin bearing thyroiditogenic epitopes. FTIR spectroscopy can be used to detect thyroiditogenic epitopes on thyroglobulin.

Keywords FTIR spectroscopy · Freshly isolated thyroglobulin · Lyophilized thyroglobulin · Thyroiditogenicity · Phenylmethylsulfonyl fluoride

1 Introduction

Thyroglobulin (Tg) is an iodinated high-molecular-weight glycoprotein, performing the functions of storing iodine and precursor of the thyroid hormones (triiodothyronine (T3)) and (thyroxine (T4)). Tg is synthesized in thyrocytes, then enters the colloid of thyroid follicles, where its tyrosyl residues are exposed to iodination in one or two positions that are adjacent to the OH functional group phenolic fragment and form monoiodotyrosine (MIT) and diiodotyrosine (DIT) residues respectively. Selected pairs of DIT residues serve as functional hormonogenic units; a coupling reaction permits a donor DIT to contribute its iodophenyl group via a quinol-ether linkage to a DIT acceptor that serves as the site of T4 formation. Analogous coupling of a MIT donor with a DIT acceptor leads to formation of T3 [1]. Thyroglobulin

✉ Igor Cherepanov
cherchem@mail.ru

[1] Laboratory of Molecular and Cell Immunology, Department of Immunology and Cell Biology, Udmurt State University, 1 Universitetskaya St, Izhevsk 426011, Russian Federation

[2] Laboratory of Biocompatible Materials, Udmurt Federal Research Center UB RAS, Izhevsk, Russian Federation

[3] Peoples′ Friendship University of Russia Named After Patrice Lumumba, Moscow, Russian Federation

[4] Department of Fundamental and Applied Chemistry, Udmurt State University, Izhevsk, Russian Federation

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re-enters the follicular cells where the thyroid hormones T3 and T4 are split from the protein backbone.

Thyroglobulin is major autoantigen to which autoreactive T lymphocytes, destroying the thyroid gland in autoimmune chronic lymphocytic (Hashimoto's) thyroiditis (HT), are directed [2–4]. Thyroglobulin is used as an antigen in ELISA kits to detect Tg autoantibodies, the presence of which in the patient's blood together with antibodies to thyroid peroxidase serve as confirmation of the diagnostic suspicion of Hashimoto's thyroiditis, as well as the primary risk factor for identifying those individuals at risk of thyroiditis [5].

In addition to being used in the clinical laboratory diagnosis of Hashimoto's thyroiditis, Tg is used for induction of experimental autoimmune thyroiditis (EAT), which is a counterpart of Hashimoto's thyroiditis, and is used to test new therapeutic agents [6, 7].

To detect anti-Tg autoantibodies and anti-Tg T cell response associated with Hashimoto's thyroiditis, as well as for the successful induction of experimental autoimmune thyroiditis, thyroglobulin carrying thyroiditogenic epitopes is necessary. The thyroiditogenic epitope is the epitope the immune response to which causes atrophy and destruction of thyroid follicles. It is known that isolated thyroglobulin easily loses thyroiditogenic epitopes [8]. The loss of thyroiditogenic epitopes is associated with Tg deiodination [8]. What other features of the Tg structure, in addition to iodine content, determine the presence of thyroiditogenic epitopes and can serve as markers of their presence, haven't been determined.

Traditionally, immunochemical assay is used to identify epitopes. However, obtaining components of the immunoassay methods is resource-intensive, requiring, among other things, the use of experimental animals. X-ray crystallography is considered to be the gold standard of epitope mapping [9]. Electron microscopy (cryo-/negative stain) [10] and surface plasmon resonance also enable to explore epitopes [11]. While different in their detail, all methods for determining epitopes rely on assessing the binding of the antibody to the antigen, and, therefore, require the production of antibodies specific to the studied epitopes.

Fourier-transform infrared (FTIR) spectroscopy serves as a valuable tool for investigating the chemical structure of proteins. The advantage of FTIR spectroscopy over the above mentioned methods is the absence of the need to obtain specific antibodies to detect epitopes.

If by means of FTIR spectroscopy it is possible to establish the features of the structure Tg bearing thyroiditogenic epitopes and distinguish Tg bearing thyroiditogenic epitopes from Tg which had lost thyroiditogenic epitopes, then this method can be used for the rapid detection of thyroiditogenic epitopes instead of immunochemical analysis.

FTIR spectroscopy was previously used to classify and biochemically characterize thyroid tissue. These studies were focused on the biochemical differences among normal and abnormal stages of thyroid diseases [12–14]. Pereira et al. characterized Tg-containing colloid of normal thyroid tissue by micro-FTIR spectroscopy [15]. Rugerry et al. examined Tg secondary structure through infrared absorption nanospectroscopy to demonstrate the method [16]. FTIR spectra of thyroid hormones have been studied [16]. FTIR spectroscopy was not used to study the structure of purified Tg, its antigenic properties, in particular thyroiditogenic properties.

2 Materials and Methods

2.1 FTIR Spectroscopy

Vibrational spectra were registered using IR-Fourier spectrometer FSM-2201 (InfraSpec Ltd, SPb, RF) equipped with the DLaTGS-detector and KBr-beamsplitter, in the wavenumber region from 4000 to 860 cm^{-1} as a transmittance spectrum at wavenumber resolution of 2 cm^{-1} for 40 scans and preliminary registration of background spectra (phosphate buffer).

Solution samples were investigated in the forms of thin films between CaF_2-windows that were assembled into infrared cell with thickness of the spacer equal of 6 μm, the instrument was purged with dry inert gas and the bend of solvent was subtracted from the initial spectra according to Surewicz et al. [17]. Strating zero-order spectra were processed with Fspec 4.3.0.9 software, the second derivative spectra after preliminary transformation from transmittance to absorbance were obtained in the same program as a result of numerical differentiation using smoothing by a fourth-order polynomial in windows up to 15 points.

The intensity of the signals of the second derivatives was estimated by the difference in the amplitudes of two adjacent extremes of the opposite sign (peak-to-peak measurement technique) [18]. This method was used to evaluate bands corresponding to MIT, DIT and secondary structural elements. An example of MIT and DIT calculations using the peak-to-peak measurement technique is presented in Fig. S1.

To calculate the relative content of the secondary structural elements, the second derivatives of the spectral bands of the amide I region were assigned to specific secondary structural elements in accordance with the literature data. The intensity of the signals was calculated using the peak-to-peak measurement technique. Then the intensity of the signals assigned to α-helix and 3_{10}-helix were summed up, and presented as α-helix. The intensity of the signals

attributed to β-sheet and β-turns were summarized and presented as β-structures. The intensity of the signals of the random coil was also calculated. The ratio of secondary structural elements types was expressed as a percentage.

2.2 Ethics Statement

Female Wistar rats were obtained from the Rappolovo breeding facility (Rappolovo, Russia). Animal experiments were performed in accordance with the ARRIVE guidelines, the U.K. Animals (Scientific Procedures) Act, 1986, and EU Directive 2010/63/EU for animal experiments. The protocol and procedures employed were ethically reviewed and approved by the Bioethics Committee of Udmurt State University (Date 01/12/2023/No. 2303).

2.3 Purification of Rat Thyroglobulin

Six rat Tg samples were purificated from the thyroids of intact rats. A column of Sephacryl S 100 26/400 equilibrated with 0.015 sodium phosphate buffer, pH 7.5 was used for this purpose, and then anion-exchange chromatography on DEAE-sepharose equilibrated with 0.015 sodium phosphate buffer, pH 7.5 was employed. The Tg were eluted using a linear NaCl gradient from 0 to 1 M. Immunoglobulin G impurity was removed from rat Tg on protein G-sepharose equilibrated with 0.015 sodium phosphate buffer, pH 7.4. In detail, the Tg purification method has been described previously [19]. The purity of Tg samples was 95%.

One portion of Tg (freshly isolated Tg) was analyzed immediately after isolation, the other was lyophilized. Three rat Tg samples were obtained by the same method, with the only change being that the homogenization of the thyroid glands of rats was carried out in the presence of a serine protease inhibitor (1 mM phenylmethylsulfonyl fluoride (PMSF) (NeoFroxx, Germany)). The isolation of Tg was performed on an AKTA pure 25 M chromatograph provided by the Center for the Collective Use of Scientific Equipment, Udmurt State University.

2.4 Immunization with Freshly Isolated and Lyophilized Tg and Assessment of Experimental Autoimmune Thyroiditis

To find out the presence of thyroiditogenic epitopes on Tg, 9–10 week-old female Wistar rats were immunized with 100 µl of emulsion containing 100 µg of freshly isolated or lyophilized rat Tg and 50 µl of incomplete Freund's adjuvant (IFA), subcutaneously, twice with an interval of 7 days. Thyroid glands were collected 6 weeks after initial immunization with Tg. Paraffin sections of thyroid were stained with hematoxylin-eosin and with FITC labeled anti-CD3 antibodies (Mybiosource, USA) in accordance with protocols previously described [19]. CD3 T lymphocytes were detected using an Olympus BX53 fluorescence microscope (Japan) provided by the Center for the Collective Use of Scientific Equipment, Udmurt Federal Research Center of the Ural Branch of the Russian Academy of Sciences. The development of autoimmune thyroiditis in rats was evaluated by the presence of T lymphocytic infiltration of the thyroid gland and atrophy and destruction of thyroid follicles [19].

2.5 Lymphocyte Proliferation Assay

Lymph node lymphocytes were obtained from rats with histologically confirmed EAT and EAT-resistant rats 6 weeks after Tg immunization. 1×10^6 cells in 300 µl of complete RPMI-1640 supplemented with 10% fetal bovine serum were stimulated with 10 ug of freshly obtained Tg or lyophilized Tg in an atmosphere of 5% CO_2 at 37 °C. After 96 h dehydrogenase activity in lymphocytic cultures was measured. WST-8 (Cell Counting Kit-8, China) assay was used.

2.6 Statistical Analysis

Statistical analysis was performed using GraphPad Prism Software. Shapiro– Wilk test for testing the normality of data was used. Statistical significance between groups was determined by paired t test. P value < 0.05 was considered statistically significant.

3 Results and Discussion

3.1 Thyroiditogenic Epitopes on Freshly Isolated and Lyophilized Rat Tg

Rat EAT model as well as human Hashimoto's thyroiditis is primarily a T-cell mediated disease [6, 7, 19–22]. The production of autoantibodies to Tg in EAT-susceptible rats is weak (Fig. S2), therefore the binding of Tg to sera obtained from rats with histologically confirmed EAT can not be used to examine the thyroiditogenic epitopes on Tg. The presence of thyroiditogenic epitopes on the freshly isolated and lyophilized rat Tg molecules was detected by the ability of Tg to cause thyroiditis in Tg-immunized rats and to induce in vitro proliferation of lymphocytes obtained from rats with histologically confirmed EAT (Fig. S3).

Immunization with freshly isolated rat Tg caused T lymphocytic infiltration of the thyroid glands (Fig. 1a), atrophy and degradation of thyroid follicles (Fig. 1b) in 4 of 8 rats. Lymphocytic thyroid infiltration and atrophic thyroid follicles are typical histopathological signs of Hashimoto's

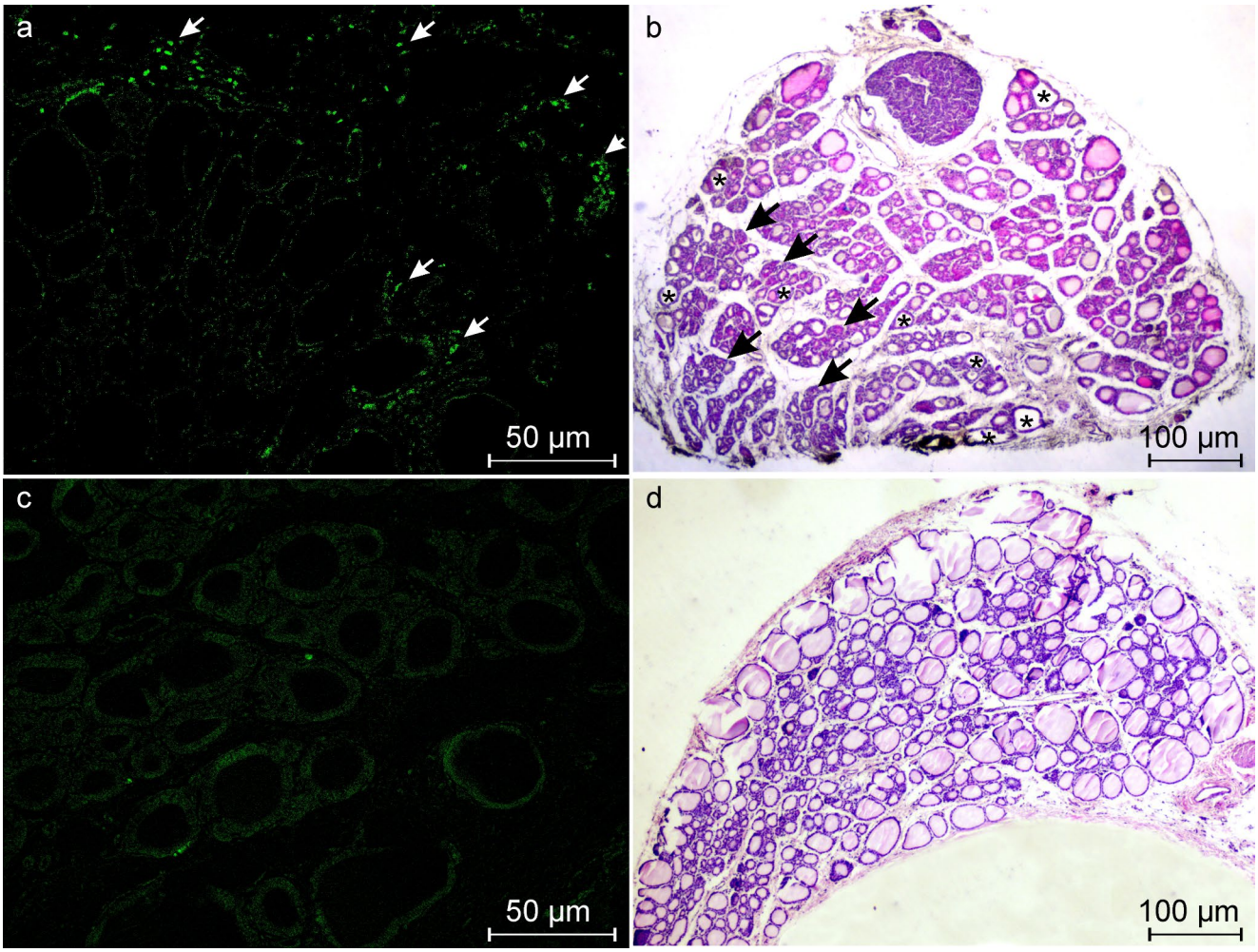

Fig. 1 Representative images of thyroid glands from rats immunized with freshly isolated Tg (**a**, **b**) and from rats immunized with lyophilized Tg (**c**, **d**). a, c. T lymphocytic infiltration in thyroids. Sections of the thyroid glands were stained with FITC labeled monoclonal antibodies against CD3 (a specific marker of T lymphocytes). **b**, **d**. Thyroids stained with hematoxylin–eosin. Black arrows - atrophic microfollicles. Asterisks—follicles devoid of colloid

thyroiditis [2–4]. Consequently, the thyroid lesions found in rats are similar to Hashimoto's thyroiditis. Immunization of rats ($n = 8$) with lyophilized rat Tg did not cause thyroid damage (Fig. 1c, d). Therefore, freshly isolated Tg carries thyroiditogenic epitopes, whereas lyophilized Tg does not.

As mentioned above the leading role in the destruction of the thyroid gland in Hashimoto's thyroiditis is assigned to T cell-mediated immune response to Tg. Therefore, to detect thyroiditogenic epitopes on Tg we studied Tg-induced proliferative response of lymphocytes obtained from lymph nodes of rats with histologically confirmed EAT. The response of lymphocytes of rats with histologically confirmed EAT to freshly isolated Tg was stronger than to lyophilized Tg (Fig. 2a). The response of lymphocytes of rats resistant to Tg-induced EAT to freshly isolated and lyophilized Tg didn't differ (Fig. 2a). Therefore, freshly obtained rat Tg carries epitopes that are the target of an autoimmune attack upon rat autoimmune thyroiditis.

3.2 Electrophoretic Analysis of Freshly Isolated and Lyophilized Rat Tg

Under dissociating conditions of electrophoretic analysis in polyacrylamide gel, the freshly isolated thyroglobulin formed major band corresponding to Mr 660 kDa and a weak band corresponding to Mr 330 kDa, indicating that the thyroglobulin is present as the whole molecules and partially as subunits thereof (Fig. 2b). Lyophilized thyroglobulin is mainly represented by subunits with Mr 330 kDa (Fig. 2b).

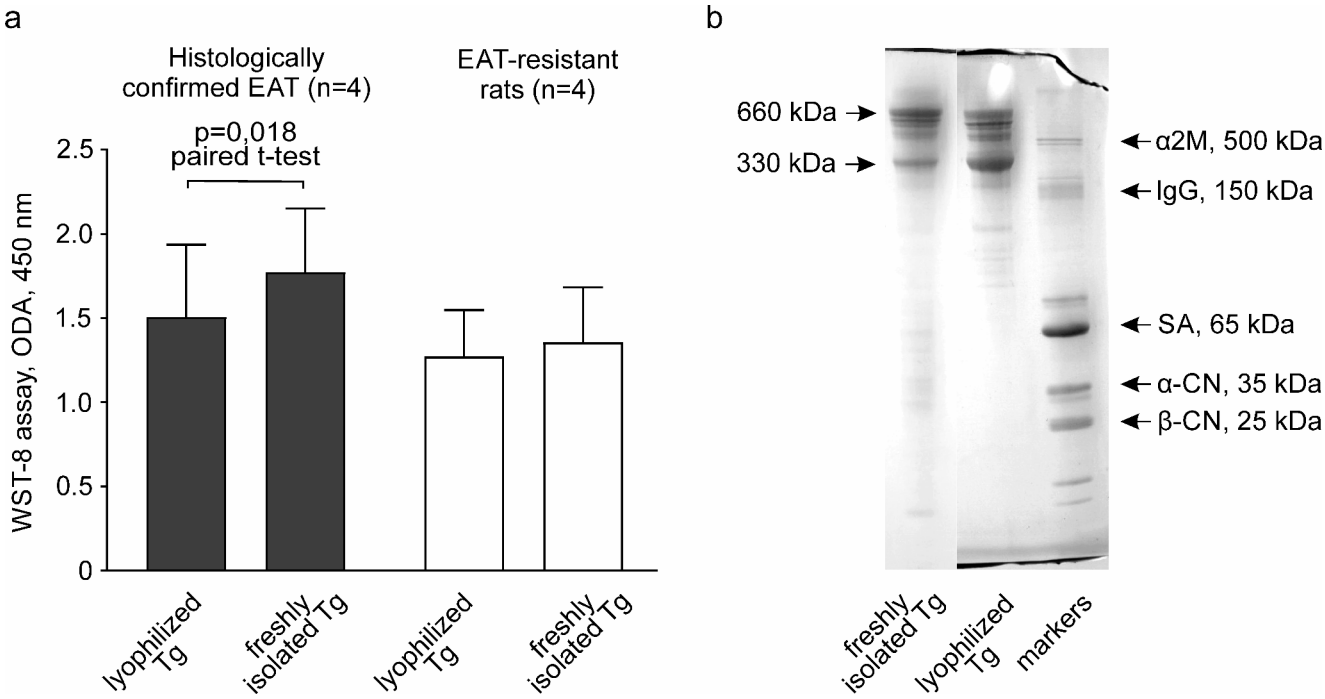

Fig. 2 **a**. Response of lymph node lymphocytes from rats with histologically confirmed EAT and EAT-resistant rats to freshly isolated and lyophilized Tg. **b**. SDS PAAG electrophoresis of rat thyroglobulin (3–15% gel)

3.3 Fourier-transform Infrared Spectra of Freshly Isolated and Lyophilized Rat Tg

Six independently produced samples of rat Tg were studied through FTIR spectroscopy. Each Tg sample was examined being freshly isolated Tg (Fig. 3a) and after lyophilization (Fig. 3b). The spectra of all the samples are characterized by good signal resolution and an even baseline, which indicates that the samples are sufficiently chemically stable. The differences between freshly isolated Tg and lyophilized Tg were observed in the region of vibrations of iodinated aromatic fragments of tyrosine and in amide I region.

3.4 Comparison of Freshly Isolated and Lyophilized Rat Tg in the Region of the Iodinated Tyrosine Vibrations

Thyroglobulin is tyrosine-rich protein. In the colloid of thyroid follicles of the thyroid gland, the tyrosine residues of thyroglobulin are iodinated and converted into MIT and DIT. When Pereira et al. analyzed spectra from the normal human thyroid tissue, the band located at 1468 cm^{-1}, they referred to the diiodotyrosine structure, whereas shoulder at 1460 cm^{-1} - to monoiodotyrosine structure and concluded that peak at 1468 cm^{-1} and its low wavenumber shoulder at 1460 cm^{-1} show the amount of iodine linked to the thyroglobulin molecule [15]. The described signals are benzoic rings vibrations (β-cycles) of tyronine [15], which is confirmed by calculations performed within the framework of density functional theory performed by Alvarez R. et al. for mono- and diiodo-substituted phenol fragments [23].

In the second derivative spectra of freshly isolated rat Tg we found a doublet - an intense peak with an extremum in the range of 1476–1470 cm^{-1} and less pronounced peak with an extremum in the range of 1466–1464 cm^{-1} (Fig. 4a, c). Based on Pereira's data [15], we assume that peaks in the range of 1476–1472 cm^{-1} may correspond to absorption of thyroglobulin diiodotyrosine, and peaks in the range of 1466–1464 cm^{-1} can be attributed to thyroglobulin monoiodotyrosine.

The samples of lyophilized rat Tg (Fig. 4b) exhibit bands centered in the interval of 1476–1474 cm-1 and bands with an extremum in the interval 1469–1466 cm^{-1} which we assigned to the β-aromatic rings of DIT and MIT, respectively. In half of the Tg samples, bands attributed to DIT were not detected after lyophilization of the protein (Fig. 4d) (Table 1).

Analysis of the intensity of peaks corresponding to the MIT and DIT structures and their ratio in Tg samples showed that in freshly isolated Tg samples carrying thyroiditogenic epitopes, DIT prevails over MIT (Table 1). In lyophilized Tg samples MIT/DIT ratio biased in favor of MIT, or is not computable because the DIT band is missing (Table 1).

Thus, the presence of thyroiditogenic epitopes on freshly isolated rat Tg is associated with the presence of a DIT band and a relatively low MIT/DIT ratio. The loss of

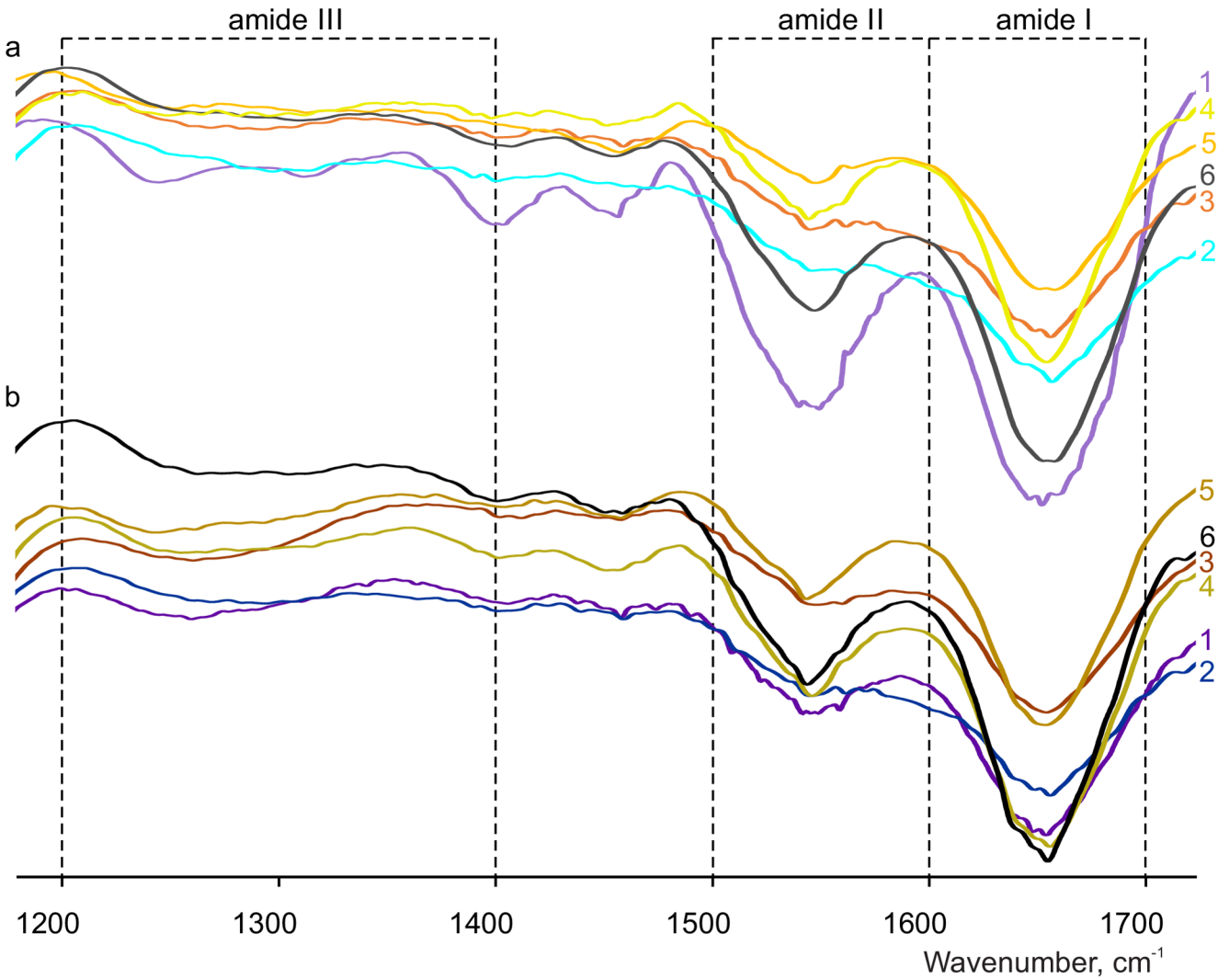

Fig. 3 Fourier-transform infrared spectra of rat Tg. **a**. Freshly isolated Tg. **b**. Lyophilized Tg

thyroiditogenic epitopes occurring after Tg lyophilization is associated with a decrease or loss of the DIT band, which is probably a consequence of Tg deiodination.

The importance of Tg iodination for preserving thyroiditogenic epitopes was previously shown by Barin et al. [8]. They found that T cell line 2D11 from NOD.H2^{h4} mouse with thyroiditis proliferated in response to normal Tg, but not to hypoiodinated Tg. Serum antibodies from NOD.H2 h4 mice with thyroiditis were poorly reactive to hypoiodinated Tg [8].

In the current study, we not only showed that the presence of thyroiditogenic epitopes on Tg is associated with the degree of its iodization, but also managed to determine the specific spectral characteristics of thyroglobulin carrying thyroiditogenic epitopes, such as the presence of vibrations of diiodotyrosine and monoiodotyrosine/diiodotyrosine relation in the range of 0.24–0.43 (95% confidence interval).

3.5 Analysis of the Secondary Structure of Freshly Isolated and Lyophilized Tg

Each major secondary structural feature of protein displays a distinct amide I frequency [24–28]. α-helices form peaks at 1650 cm^{-1} and 1655 cm^{-1} [26, 28], 3_{10}-helix form peaks at 1666 cm^{-1} [24], 1657 cm^{-1} [25], 1663 cm^{-1} [27], while β-structures (β-sheets) according to various authors (scientists) show bands at 1616 cm^{-1} and at 1635 cm^{-1} [24], bands in the interval 1610–1639 cm^{-1} and 1689–1695 cm^{-1} [25], β-structures (β-turns) form bands with highs at 1680 cm^{-1} and 1695 cm^{-1} [24], 1673 cm^{-1} and 1681 cm^{-1} [25]. Peaks for unordered structures occur at 1640–1649 cm^{-1} [25], 1647 cm^{-1} [25] and 1648 [27].

Figure 5 shows assignment of amide I region bands to the secondary structural elements in the one of the Tg samples. In order to determine the contribution of the secondary

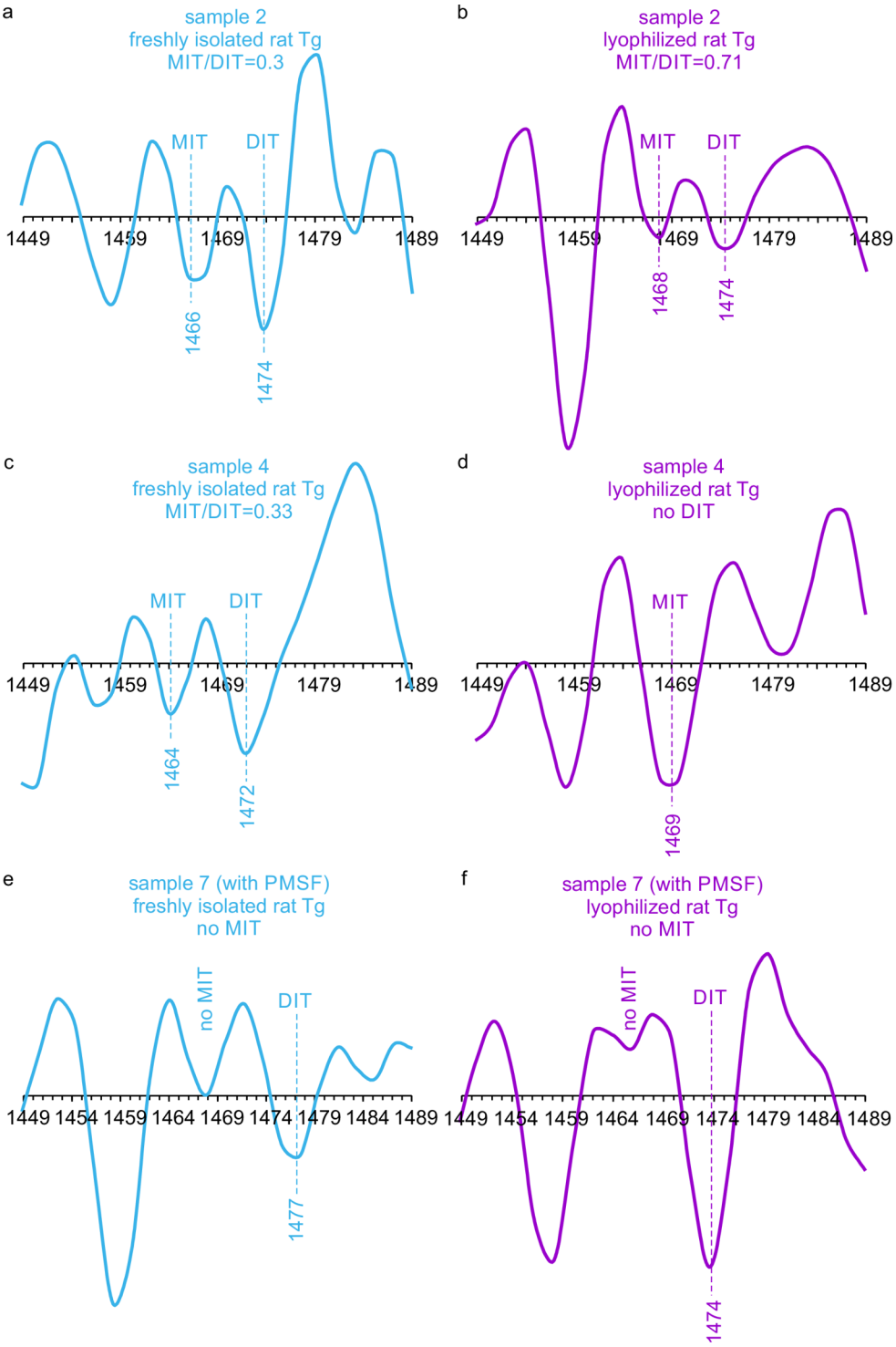

Fig. 4 Rat Tg in the region of the iodinated tyrosine residues. Representative images. **a**, **c**. Freshly isolated Tg. **b**, **d**. Lyophilized Tg. **e**, **f**. Tg isolated in the presence of phenylmethylsulfonyl fluoride

structural elements in protein structures, the ratio of signal intensities of the second derivatives of the spectral bands of the amide I region is calculated.

The proportion of different type of the secondary structures in each of the studied Tg samples and comparison of the secondary structure compositions of freshly obtained Tg and lyophilized Tg are presented in Table 2. It follows from Table 2 that in the secondary structure of lyophilized thyroglobulin samples without thyroiditogenic epitopes compared with the secondary structure of freshly isolated thyroglobulin carrying thyroiditogenic epitopes, the proportion of alpha helices is significantly less (Paired t test, $p = 0.003$). Consequently, the loss of thyroiditogenic epitopes is associated with a change in

Table 1 MIT/DIT ratio in freshly isolated and lyophilized tg samples

Tg sample, #	Freshly isolated	Lyophilized
1	0.45	0.61
2	0.30	0.71
3	0.29	DIT vibration is not revealed
4	0.33	DIT vibration is not revealed
5	0.21	DIT vibration is not revealed
6	0.43	0.75
mean±SD	0.035±0.09	N/a
95% confidence interval	0.24– 0.43	N/a
7	MIT vibration is not revealed	MIT vibration is not revealed
8	0.8	MIT vibration is not revealed
9	1.22	MIT vibration is not revealed
mean±SD	N/a	N/a

the secondary structure of the protein, namely, the random transition of alpha helices into betta structures or an unordered tangle (Table 2).

The proportion of alpha helices, beta layers and a random coil in the freshly isolated rat Tg molecule (Table 2) agrees well with the data of Formisano et al., who calculated the secondary structure of native rat thyroglobulin, derived from its experimental circular dichroism spectrum [29]. Formisano et al. showed that native rat thyroglobulin contains 32% alfa-helix, 35% beta-sheet, 13% beta-turn and 20% random structures [29].

Studies of the relationship between MIT/DIT ratio and the proportion of alpha helices in Tg have revealed a negative correlation between them (k= -0.8, $p = 0.009$).

According to the literature, the iodine content and the secondary structure of thyroglobulin are related. Pereira et al. have showed, that non-iodinated form of human thyroglobulin has a β-sheet conformation, while the iodinated form of thyroglobulin has an α-helical conformation

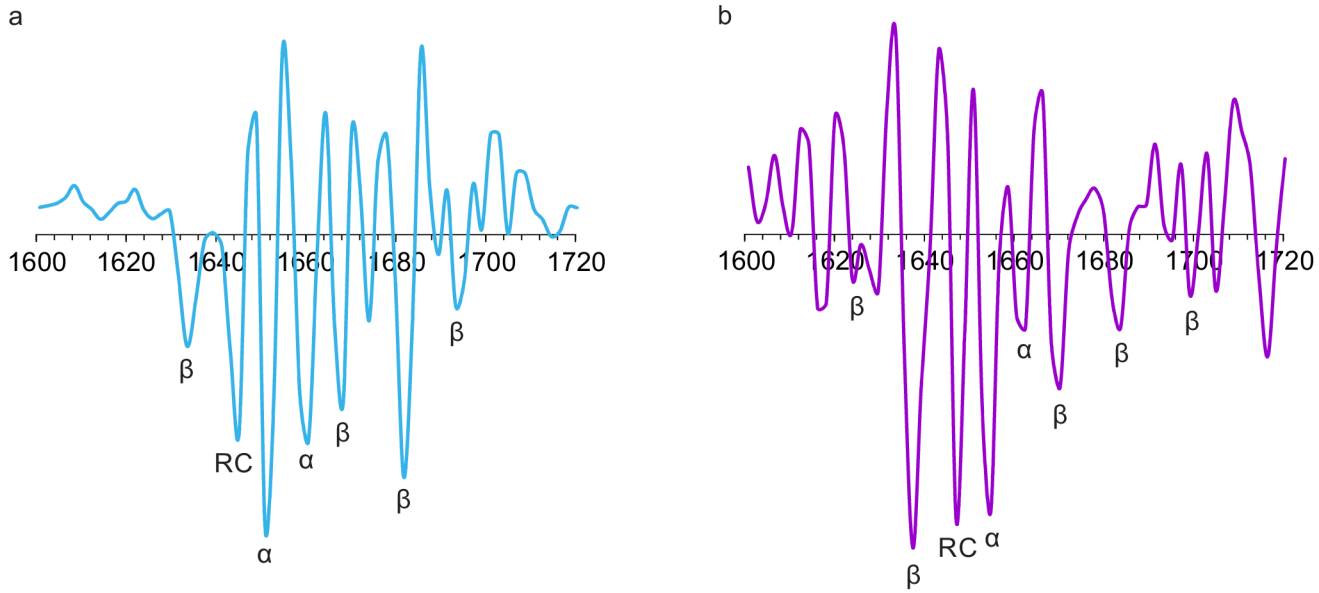

Fig. 5 The second derivatives of the Tg spectrum in the amide 1 region and their interpretation. **a**. Freshly isolated Tg. **b**. Lyophilized Tg. α– the band corresponding to the secondary structure represented by the alpha helix; β– the band corresponding to the secondary structure represented by beta structures (β-sheets and β-turns); RC - a band corresponding to the thyroglobulin structure represented by random coil

Table 2 Secondary structure of freshly isolated and lyophilized tg

Freshly isolated Tg				Tg after lyophilization		
Sample #	alpha helices	beta sheets	random coil	alpha helices	beta sheets	random coil
1	39	46	15	26	54	20
2	37	63	0	24	57	19
3	39	48	13	10	75	15
4	32	67	1	22	65	13
5	33	39	28	19	74	7
6	36	64	0	23	65	12
Mean±SD	36±3	54±12	10±11	21±6*	65±9	14±5
* - Paired t test, p=0.003						
7	22	75	3	21	71	8
8	20	56	24	18	51	31
9	30	55	15	13	61	26
Mean±SD	25±5	62±9	13±9	19±5	65±11	16±15

[15]. Data of Formisano et al. can explain this relationship. It was shown that areas of high amounts of iodine are located in the part of the molecule having α-helical conformation [29].

Thus, the markers indicating the presence of thyroiditogenic epitopes on Tg are not only the presence of vibrations of DIT, but also relatively high alpha-helix content. The loss of thyroiditogenic epitopes on Tg is associated with a weakening or complete disappearance of DIT oscillations and a decrease in the proportion of alpha helices in the secondary structure.

3.6 Analysis of Rat Thyroglobulin Isolated under Conditions Inhibiting Proteolysis

There is evidence that Tg isolated by several procedures remain contaminated by endogenous proteases, that degrade Tg. In this regard, when the structure of Tg is examined, its extraction is recommended to be carried out under conditions inhibiting proteolysis [30]. Therefore, we have extracted some of the Tg samples (# 7–10, Table 2) in the presence of phenylmethylsulfonyl fluoride (PMSF)– serine protease inhibitor, which is widely used in the isolation of proteins from tissues.

Both freshly isolated and lyophilized samples of thyroglobulin, purified with PMSF added, are characterized by a high MIT/DIT ratio (Table 1), or a complete absence of fluctuations related to MIT (Table 1; Fig. 4e, f). MIT residues together with DIT residues in normal Tg are known to form a precursor of triiodothyronine. Therefore, the loss of MIT indicates a loss of Tg nativity. Besides, Tg samples, obtained under conditions inhibiting proteolysis, is characterized by low proportion of alpha helices (20–30%), not only after lyophilization, but also after being freshly isolated (Table 2). The proportion of alpha helices in the structure of freshly isolated Tg carrying thyroiditogenic epitopes is more than 32% (Table 2). A low proportion of alpha helices is typical for Tg without thyroiditogenic epitopes (Table 2). Therefore, we assume that Tg purified in the presence of PMSF could lose thyroiditogenic epitopes.

Thus, thyroglobulin isolated under conditions inhibiting proteolysis loses iodinated tyrosine residues and part of the alpha helices, which are a characteristic feature of Tg bearing thyroiditogenic epitopes. Therefore, PMSF is not suitable for extraction of native Tg, bearing thyroiditogenic epitopes.

4 Conclusion

FTIR spectroscopy can be used to detect thyroiditogenic epitopes on Tg. The markers indicating the presence of thyroiditogenic epitopes on Tg are the presence of vibrations of DIT in the wavenumber range of 1476–1470 cm^{-1}; MIT/DIT relation in the range of 0.24–0.43 (95% confidence interval) and high (≥ 32%) alpha-helix content. The loss of thyroiditogenic epitopes on Tg is associated with a weakening or complete disappearance of DIT oscillations and a decrease in the proportion of alpha helices in the secondary structure. Tg extracted with phenylmethylsulfonyl fluoride (PMSF) added is characterized by the same relatively high monoiodotyrosine/diiodotyrosine relation and low proportion of alpha helices as thyroglobulin without thyroiditogenic epitopes. Therefore, serine protease inhibitor PMSF is not suitable for extracting native Tg bearing thyroiditogenic epitopes.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10930-024-10243-8.

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Author Contributions I.C., A.S. and L.B. contributed to the study methodology. L.B. contributed to the study conception. I.C., A.S., L.B., A.T., D.M., T.H. and P.I. conducted experiments. I.C., L.B. and A.S wrote the manuscript. I.M. reviewed and edited the text. All authors contributed to the analysis and interpretation of the data. All authors read and approved the final manuscript.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing Interests The authors declare no competing interests.

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