

Stearyl poly(ethylene oxide) grafted surfaces for preferential adsorption of albumin

Part 2. The effect of molecular mobility on protein adsorption

Jian Ji*, Linxian Feng, Yongxin Qiu, Xiaojie Yu

Department of Polymer Science and Engineering, Zhejiang University, HangZhou 310027, People's Republic of China

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Abstract

The adsorption of plasma proteins (fibrinogen and albumin) from buffer onto polystyrene-graft-(stearyl-poly(ethylene oxide)) (PS-g-SPEO) was investigated by radioiodine labeling of proteins using the iodine monochloride method. The molecular mobility of poly(ethylene oxide) grafts was determined by ESR spectroscopy, and the effects of PEO mobility on the ability of PS-g-SPEO to resist the non-specific protein adsorption have been studied. The non-specific adsorption of fibrinogen was found to decrease with increasing PEO mobility, whereas the interfacial energies lack significant correlation to the protein adsorption. The elastic restoring force seems much more important than hydrophobic interaction in PS-g-SPEO/protein interfaces. The high molecular mobility of PEO side chain is supposed to be the key effect on the protein-resistant properties of PS-g-SPEO. The protein adsorption results also reveal that the molecular mobility of PEO has a quite important role in developing albumin preferential materials. A high albumin preferential adsorption layer has been developed by immobilizing the stearyl end groups onto high mobile PEO chains. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Molecular mobility; Protein adsorption; Blood compatibility

1. Introduction

Polymeric biomaterials have been widely used in both medical and pharmaceutical applications, and contribute significantly to the quality and effectiveness of health care systems [1]. However, like other materials, polymers exposed to blood generally adsorb a layer of protein on their surfaces very rapidly. The composition of the protein layer varies with the surface properties of the materials and usually induces thrombosis and infection [2].

An important trend in blood contact material research is developing a polymer system that combines capabilities of biological recognition (biomimetic) with the special physicochemical properties of the synthetic polymer system [3]. As a myriad of molecular plasma constituents, including probably more than 200 proteinaceous components, compete to differing degrees at material interfaces, the general principle in the design of surface to trigger a specific biological pathway is that non-specific interactions should be inhibited [4].

A series of polystyrene-graft-(stearyl-poly(ethylene oxide)) (PS-g-SPEO), which have both long poly(ethylene oxide) side chains and stearyl (C-18) end groups, have already been synthesized in our laboratory [5]. The stearyl end group is expected to be able to bond albumin preferentially by the specific interaction between the C-18 chain and the alkyl-binding site of albumin [6,7]. The advantages of using poly(ethylene oxide) are its low interfacial energy, its non-adhesive property and the high dynamic motion which reduces the non-specific protein adsorption [8]. It presumes here that an albumin preferential adsorption surface can be developed by immobilizing the stearyl end groups onto high mobile PEO chains.

The PEO mobility, which contributes to the steric exclusion, plays an extremely important role in the ability of PEO to reduce non-specific protein adsorption [9]. However, difficulties are involved in determining the PEO mobility at polymer/water interface and studies on the effect of PEO mobility on protein adsorption have been quite scarce.

A method highly sensitive to PEO mobility at polymer/water interface has been found in our lab [10]. In this study, the protein adsorption onto PS-g-SPEO was investigated and correlated with both the interfacial energy and the PEO mobility.

*Corresponding author. INEB-mat, Rua do Campo Alegre 823, 4150 Porto, Portugal. Fax: +351-609-4567.

E-mail address: Jian@ibmc.up.pt (J. Ji).

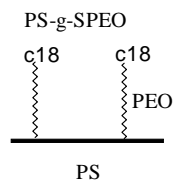


Fig. 1. Schematic structure graph of PS-g-SPEO copolymer.

2. Experimental

2.1. Materials

The stearyl-poly(ethylene oxide) (SPEO) grafted modeling polymers (PS-g-SPEO) were synthesized by free radical polymerization of styrene with acryloyl SPEO macromonomer. The copolymers obtained exhibit the expected molecular structures as indicated (see Fig. 1) [5].

2.2. Surface characterization of PS-g-SPEO

2.2.1. X-ray photoelectron spectra

Samples for analysis by X-ray photoelectron spectra (XPS) were in the form of polymer films coated on flat aluminum substrates. The films were prepared from a solution in chloroform (approximately 0.5% w/v) by dip coating, drying overnight in vacuum at ambient temperature. Spectra were recorded on an ESCALAB MARK spectrometer by using AlK_{α} exciting radiation. Spectra were obtained in digital form. Curve fitting of the spectra was accomplished using a non-linear least square method. A Gaussian–Lorentzian function was assumed for the curve-fitting process [11].

2.2.2. Contact angle determination

Water contact angle measurements were carried out using the captive air bubble techniques developed by Good and Neumann [12]. The films for contact angle measurement were prepared by dip coating from a 0.5% (w/v) solution of the polymer in chloroform onto clean glass substrates and dried overnight in vacuum at room temperature. The surfaces were equilibrated with doubly distilled water for 24 h prior to the collection of the surface–water–air and surface–water–octane static bubble contact angle. A minimum of 10 measurements was used to calculate the average contact angle in each condition.

2.2.3. ESR measurements

ESR spectroscopy to determine the molecular mobility of PEO grafts in PS-g-SPEO has been described in detail previously [10]. Polymer films were prepared by solution casting from a 0.5% (w/v) solution of the copolymer in chloroform onto clean glass substrates and dried overnight in vacuum at room temperature. 1,4-Hydroxyl-TEMPO (Adrich) was chosen as the spin probe. ESR samples were prepared by immersing the polymer films on a 10^{-4} mol l^{-1}

solution of 1,4-hydroxyl-TEMPO in water. Samples were maintained at room temperature for 24 h before washing extensively to remove any surface-bound spin probe. ESR measurements were made on a JES-Fixing (JEOL) spectrometer. Samples were introduced into the cavity of a cylindrical glass tube, diameter 5 mm. Spectra were recorded using the following instrumental parameters: scan range 250 G; time constant 0.3 s; scan time 4 min; modulation amplitude 1 G; microwave power 4 mW; each sample being measured 3–5 times for reproducibility. The parameters of the ESR spectra were measured in comparison with those of Fremy's salt. (nitrogen hyperfine constant $\alpha_N = 13.0$ G). The rotational correlation time τ_c was calculated with the following formula:

$$\tau_c = 6.51 \times 10^{-10} \times \Delta H(0) \times \{ [h(0)/h(-1)]^{1/2} + [h(0)/h(1)]^{1/2} - 2 \} \quad (1)$$

where $\Delta H(0)$ is the line width (in G) of the central line, $h(0)$, $h(-1)$ and $h(+1)$ are the peak heights of $m_1 = -1, 0$ and $+1$ derivative lines, respectively.

2.2.4. Protein adsorption measurement

Human proteins were obtained commercially: Human serum albumin was from Sigma and was 97% electrophoretically pure. Human fibrinogen was also from Sigma and was 95% clottable.

Albumin and fibrinogen were labeled by ^{125}I using the iodine monochloride technique as described by Brash [13]. Experiments were conducted to verify no dependence of the measured surface concentration on percent labeled protein over a range of 1–20% in our lab. Amounts of ^{125}I -labeled protein were added to buffer to give the mixtures containing 5% labeled and 95% unlabeled protein.

Surfaces for protein adsorption were prepared by solution coating from a 1% (w/v) solution of the polymer in chloroform on the insides of glass tubes (diameter 3 mm). All surfaces were dried overnight in vacuum at room temperature and were characterized by microscope examination to confirm no flaw of the polymer membrane. All surfaces were equilibrated with tris–HCl (0.05 mol l^{-1} , pH 7.4) buffer for 24 h prior to protein adsorption measurements.

The tubes were filled initially with tris–HCl buffer. This was then displaced by 10 times (by volume) protein solution to confirm that no air–solution interface had made contact with the test surface. After the appropriate contact time, the solution was displaced similarly by 30 times (by volume) buffer. All adsorption measurements were carried out at 25°C. Surface radioactivity was measured by a γ -counter and converted to mass of protein per cm^{-2} by comparison with an aliquot of protein solution.

At least five experiments were conducted for each set of conditions. Standard deviation based on all measurements considered being at steady-state range from $\pm 10\%$ of the mean at low surface concentration to $\pm 5\%$ at high surface concentration.

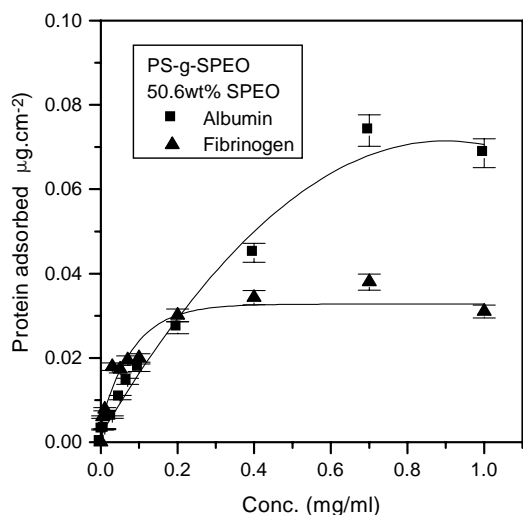


Fig. 2. Protein adsorption from single protein buffer onto PS-g-SPEO. Samples were exposed to ^{125}I -labeled albumin or ^{125}I -labeled fibrinogen solution at 25°C , 3 h. The samples were then washed by 30 times volume buffer and counted in γ -counter. Each point represents the average of five experiments with standard deviation from 2.0 to 5.0% within each set.

Curve fitting of the protein adsorption isotherm and kinetics (Figs. 2 and 3) was accomplished using software from Microcal Origin[®]. A Langmuir function was used for curve fitting and obtaining the plateau value shown in Tables 2 and 3.

3. Results

3.1. Surface characterization of PS-g-SPEO

The surface chemical composition of the PS-g-SPEO copolymers was studied by XPS. As shown in Table 1, the PS-g-SPEO copolymers have a much higher value of SPEO surface density than the bulk composite.

Data from captive bubble experiments, in which the surface was incubated with water for 24 h, are shown in Table 1. The interfacial free energies of the polymer with water, γ_{sw} , the polar component and the depolar component of the interfacial free energies, $\gamma_{\text{sv}}^{\text{p}}$, $\gamma_{\text{sv}}^{\text{d}}$, were calculated according to the following equation proposed by Andrade et al. [14].

$$\gamma_{\text{sw}} = \gamma_{\text{sv}}^{\text{p}} + \gamma_{\text{sv}}^{\text{d}} - 72.1 \cos \theta_{\text{Air}} \quad (2)$$

$$\gamma_{\text{sv}}^{\text{p}} = \frac{50.5k_1}{50.5 - k_1} \quad (3)$$

$$\gamma_{\text{sv}}^{\text{d}} = \frac{21.6[k_2 - 50.5\gamma_{\text{sv}}^{\text{p}}/(50.5 + \gamma_{\text{sv}}^{\text{p}})]}{21.6 - k_2 + 50.5\gamma_{\text{sv}}^{\text{p}}/(50.5 + \gamma_{\text{sv}}^{\text{p}})} \quad (4)$$

where $k_1 = 50.5(1 + \cos \theta_{\text{Octane}})/4$; and $k_2 = 72.1(1 + \cos \theta_{\text{Air}})/4$.

As shown in Table 1, the copolymer/water interfaces usually have high γ_{sw} and $\gamma_{\text{sv}}^{\text{d}}$. This may have arisen from

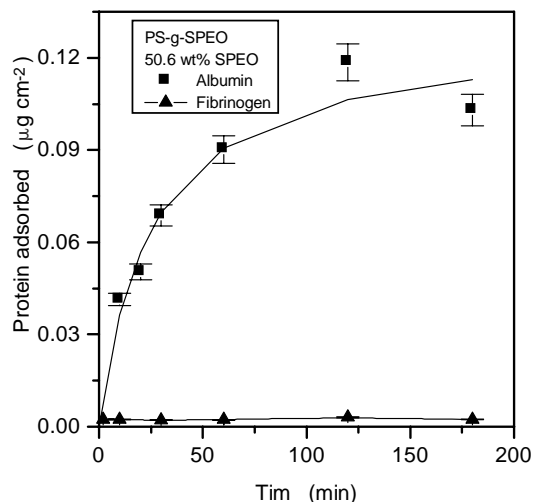


Fig. 3. Protein competitive adsorption from multi-component buffer onto PS-g-SPEO. Samples were exposed to ^{125}I -labeled albumin/fibrinogen or ^{125}I -labeled fibrinogen/albumin mixture (fibrinogen 0.03 mg ml^{-1} , albumin 0.4 mg ml^{-1} , with a mole ratio, fibrinogen to albumin of 0.015 (i.e. near to values found in plasma.) for appropriate time, at 25°C . The samples were then washed by 30 times volume buffer and counted in γ -counter. Each point represents the average of five experiments with standard deviation from 5.0 to 7.0% within each set.

the effect of the hydrophobic stearyl end group. It is a striking observation that the PS-g-SPEO-72.6, the surface with a depth of at least 5 nm is fully occupied by SPEO, has lower interfacial free energies of the polymers with water γ_{sw} .

By dispersing the stable organic radicals into the polymer surface with a 4-hydroxyl-Tempo aqueous solution, the PEO molecular mobility in the PS-g-SPEO/water interfaces was evaluated with the help of the rotational correlation time of a spin probe calculated from ESR spectroscopy [10]. Two parameters have been thoroughly investigated. The nitrogen superfine constant (α_{N}) value of the probe, which is highly sensitive to local polarity, was used to check possible association of the probe. The results (Table 2) show that α_{N} values in PS-g-SPEO copolymer are $16.53 \pm 0.23 \sim 16.69 \pm 0.20 \text{ G}$, which correspond to the probe in PEO, whereas the α_{N} in PS is $26.16 \pm 0.53 \text{ G}$. The hydrophilic spin probe is strongly biased to the PEO phase of the PS-g-SPEO membranes. The rotational correlation time τ_{c} , which is viewed as the time taken for an axis of the nitroxide group to travel through one rad, can be employed to assess molecular motion in the PS-g-SPEO/water interface [15].

It is of interest to examine that the τ_{c} does not always decrease with increasing surface density of SPEO, it passes a minimum at PS-g-SPEO-50.6.

3.2. Protein adsorption from protein buffer onto PS-g-SPEO

Protein adsorption onto PS-g-SPEO from single-component protein buffer is shown in Fig. 2. The adsorption

Table 1
Static water contact angle data for PS-*g*-SPEO

Sample	Side chain M_n	SPEO ^a (wt%)	θ_{Octane}^b	θ_{Air}^b	$\gamma_{\text{sv}}^p{}^c$	$\gamma_{\text{sv}}^d{}^c$	γ_{sw}^c
PS		–	148	80	1.99 ± 0.21	175.81 ± 11.87	165.29 ± 10.73
PS- <i>g</i> -SPEO-72.6 ^d		100	64.3	47.6	28.22 ± 0.67	27.41 ± 0.06	6.99 ± 0.42
PS- <i>g</i> -SPEO-50.6	SPEO 1.7 K	84.1	92.3	51.9	15.96 ± 0.64	80.45 ± 0.07	51.90 ± 0.90
PS- <i>g</i> -SPEO-31.0		69.0	93.5	53.3	15.48 ± 0.60	78.95 ± 0.51	51.29 ± 1.23
PS- <i>g</i> -SPEO-24.7		64.8	85.4	47.6	18.68 ± 0.62	70.81 ± 0.08	40.84 ± 0.76

^a Surface density of the SPEO, calculated by ESCA.

^b Polymer–water–Air and Polymer–water–air at 298 K, mean value ($n = 10$) is given in degrees, standard deviations less than ±2°.

^c Interfacial energy data are given in erg cm⁻².

^d The X in PS-*g*-SPEO- X indicates the bulk density of the SPEO in wt%, calculated by means of first derivative UV spectrometry [5].

amount increases and appears to reach a plateau value with protein concentration increasing.

From a Langmuir adsorption fitting, the plateau values from adsorption isotherms are shown in Table 3. The trend appears to be that PS-*g*-SPEO adsorbs more albumin than fibrinogen. It is of interest to note that the non-specific adsorption of fibrinogen onto PS-*g*-SPEO also does not always decrease with increasing surface density of SPEO (Table 3), the amount of absorbed protein passes a minimum at sample PS-*g*-SPEO-50.6. Sample PS-*g*-SPEO-72.6 (the surface with a depth of at least 5 nm is fully occupied by SPEO) has rather a high level of fibrinogen adsorption.

Data for the competitive adsorption of the ¹²⁵I-labeled fibrinogen and albumin (or ¹²⁵I-labeled albumin and fibrinogen) buffer is shown in Fig. 3.

From a Langmuir adsorption fitting, the surface concentrations (Γ_{eq}) are shown in Table 3. Compared with PS, PS-*g*-SPEO copolymers exhibit a very low fibrinogen adsorption and high levels of absorbed albumin. The ratio of Γ_{eq} for albumin to that for fibrinogen, $\Gamma_{\text{Alb/Fib}}$, can be used to indicate the preference of protein adsorption. Again, the preference of protein adsorption does not increase with increasing surface density of SPEO. A highly albumin preferential adsorption surface is formed, with the ratio of albumin to fibrinogen of 51.5, at PS-*g*-SPEO-50.6.

4. Discussion

Based on the strategy of engineering the surface for specificity, a lot of investigators have been stimulated to immobilize the specific ligand onto the surface. As the body fluid contains large numbers of structurally fragile protein molecules, which will compete at the surface, the resistance to non-specific interaction is as important as improving specific interaction for developing a high specific protein layer. The alkylation of polymer with C-18 linear alkyl chain, due to the specific interaction between the C-18 chain and the alkyl-binding site of albumin, has been shown to increase the albumin adsorption at polymer/blood interfaces [6,7]. However, the high albumin preferential layer is not obtained by direct covalent binding of the C-18 chain to the surface. The results of protein adsorption presented here show that when the stearyl is immobilized on the polystyrene via a PEO spacer, the stearyl end group can still bond albumin preferentially, while the flexible, hydrophilic PEO side chains resist fibrinogen effectively. A highly albumin preferential adsorption surface is formed, with the ratio of albumin to fibrinogen of 51.5, at PS-*g*-SPEO-50.6.

It is of interest to examine the present data in more detail with respect to the influence of PEO mobility on the non-specific fibrinogen adsorption. The adsorption of fibrinogen as a function of interfacial energy is shown in Fig. 4. No

Table 2
Structural parameters, α_N , τ_c of PS-*g*-SPEO copolymer

Sample code	Side chain M_n	SPEO (wt%) ^a	α_N^b (G)	τ_c^b (ns)
PS	–	–	26.16 ± 0.53 ^c	–
PEO	2000	100	16.70 ± 0.20	–
PS- <i>g</i> -SPEO-72.6 ^d		100.0	16.67 ± 0.21	1.07 ± 0.05
PS- <i>g</i> -SPEO-50.6	SPEO(1700)	84.1	16.69 ± 0.19	0.30 ± 0.04
PS- <i>g</i> -SPEO-31.0		69.0	16.69 ± 0.20	0.46 ± 0.03
PS- <i>g</i> -SPEO-24.7		64.8	16.53 ± 0.23	1.93 ± 0.06

^a Surface density of the SPEO, calculated by ESCA.

^b The mean value ($n = 3$) of α_N , τ_c .

^c Calculated according to the formula: $\alpha_N = 1/3(2T_{\perp} + T_{\parallel})$.

^d The X in PS-*g*-SPEO- X indicates the bulk density of the SPEO in wt%, calculated by means of first derivative UV spectrometry [5].

Table 3
Structural parameters, protein adsorption on PS-*g*-SPEO copolymer

Sample code	SPEO ^a (wt%)	Γ^b ($\mu\text{g cm}^{-3}$)		Γ_{eq}^c ($\mu\text{g cm}^{-3}$)		$\Gamma_{\text{Fib/Alb}}^d$
		Fib	Alb	Fib	Alb	
PS	0.0	1.00	0.140	0.115	0.085	0.739
PS- <i>g</i> -SPEO-72.6 ^e	100.0	0.10	0.360	0.007	0.178	25.4
PS- <i>g</i> -SPEO-50.6	84.1	0.031	0.070	0.002	0.103	51.5
PS- <i>g</i> -SPEO-31.0	69	0.070	0.166	0.068	0.172	2.53
PS- <i>g</i> -SPEO-24.7	64.8	0.16	0.25	0.160	0.215	1.34

^a Surface density of the SPEO, calculated by ESCA.

^b Surface plateau adsorption amount of protein adsorption isotherms from single buffer at 3 h, 25°C. Each point represents the average of five experiments with standard deviation from 2.0 to 5.0% within each set.

^c Surface equilibrated adsorption amount of protein adsorption kinetics from multi-component buffer (fibrinogen 0.03 mg ml⁻¹, albumin 0.4 mg ml⁻¹). Each point represents the average of five experiments with standard deviation from 5.0 to 7.0% within each set.

^d The ratio of Γ_{eq} for albumin to that for fibrinogen, $\Gamma_{\text{Alb/Fib}}$, can be used to indicate the preference of protein adsorption.

^e The X in PS-*g*-SPEO-X indicates the bulk density of the SPEO in wt%, calculated by means of first derivative UV spectrometry [5].

significant relationship between the fibrinogen adsorption and the interfacial free energy is evident.

In Fig. 5, however, the PEO mobility at PS-*g*-SPEO/water interfaces corresponds very well with the amount of fibrinogen absorbed. PS-*g*-SPEO absorbs less fibrinogen with increasing EO segment mobility (τ_c decreasing).

Some researches have reported that PEO-based polymers generally have surfaces at which hydrophilic PEO chains are expanding into water [16,17]. The protein-resistant character of these PEO chains is generally recognized as a steric exclusion effect. de Gennes [9] has studied the physics of terminally attached PEO surface theoretically, and explained that the steric exclusion effect could be attributed to osmotic pressure and elastic restoring. The high mobile surface leads to stronger elastic restoring force. At the polymer/protein solution interface, the steric exclusion effect competes with the van der Waals attraction and the

hydrophobic interaction, and influences strongly the protein adsorption.

The results above suggest that the elastic restoring force seems much more important than the hydrophobic interaction in the PS-*g*-SPEO/protein interface. The high surface mobility of SPEO side chain maybe the key effect on the protein-resistant properties of PS-*g*-SPEO. And the highest albumin preferential adsorption surface is formed at PS-*g*-SPEO-50.6 with the highest PEO mobility. The effect of PEO mobility on the ability of PEO to reduce non-specific protein adsorption has quite an important influence on the albumin preference of PS-*g*-SPEO.

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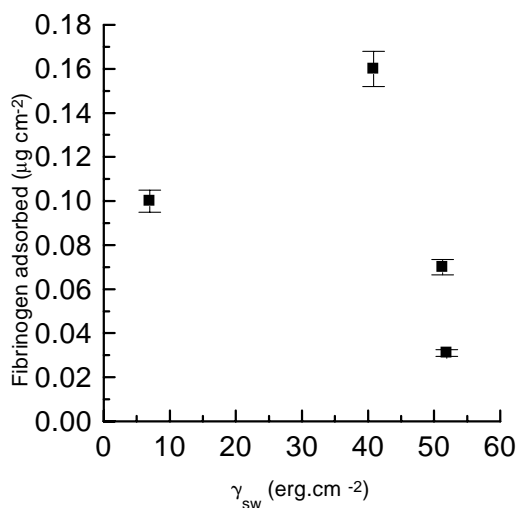


Fig. 4. Fibrinogen adsorption as a function of interfacial free energy of the polymer with water.

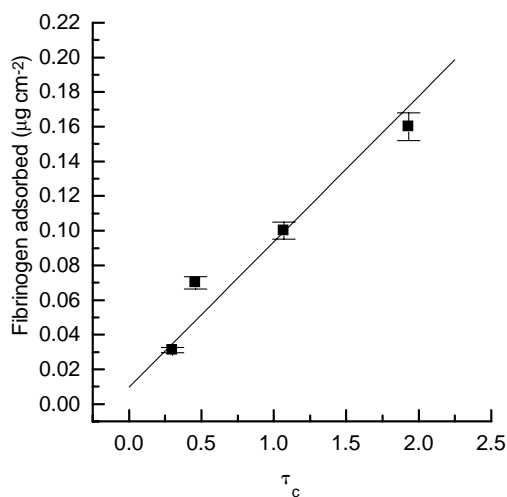


Fig. 5. Fibrinogen adsorption as a function of the τ_c , which indicated the PEO mobility of the PS-*g*-SPEO copolymer (The higher mobility of PEO corresponds to lower τ_c).

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