

Spectroscopic Study of the Formation of Ternary Complex, Starch/Soy Protein Isolate/Polyacrylic Acid

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Abstract: The formation process of a non-covalent ternary complex comprising soy protein isolate (SPI), soluble starch, and polyacrylic acid (PAA) in aqueous solution was studied based on fluorescence, ultraviolet (UV) absorption, and resonance light scattering (RLS) spectra and the thermal stability of the complex was also investigated. The fluorescence results indicated that SPI and starch could interact with PAA, and the corresponding apparent binding constants were 2.02×10^5 L/mol and 2.16×10^3 L/mol, respectively. The addition of PAA to SPI/starch solution not only resulted in changes of fluorescence intensity and the maximum emission wavelength but also generated an apparent common fluorescence emission wavelength, which demonstrated the formation of a new ternary complex. UV and RLS experiments indicated that SPI, starch, and PAA were the basic structural units to produce the ternary complex, and were conjugated through interactions among each other. Moreover, the thermal stability of the ternary complex was investigated by RLS technology and the corresponding kinetic parameters of thermal degradation were calculated based on RLS data, thereby confirming the formation of the ternary complex and the role of PAA in the process of complex formation.

Key words: ternary complex; formation; spectroscopy

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淀粉/聚丙烯酸/大豆蛋白三元复合物形成过程的光谱学研究

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摘要: 基于荧光光谱和共振光散射光谱 (RLS) 并结合 UV 紫外光谱研究了可溶性淀粉、大豆分离蛋白 (SPI) 以及聚 (丙烯酸) (PAA) 在溶液中形成三元非共价复合物的过程, 并考察了其热稳定性能。荧光实验结果表明 SPI 和淀粉可以分别与 PAA 发生相互作用, 其相应的表观结合常数分别为 2.02×10^5 和 2.16×10^3 L/mol。当 PAA 加入到 SPI / 淀粉溶液, 不仅会导致体系最大荧光发射波长和荧光强度的变化, 而且也产生一个较为明显的荧光等同发射点, 这表明三者之间形成了一个新的三元复合物。UV 和 RLS 的实验结果也说明了淀粉、聚丙烯酸以及大豆蛋白是形成三元复合物的基本结构单元, 并通过彼此间的相互作用结合在一起。此外, 通过 RLS 技术研究了三元复合物的热稳定性, 并基于 RLS 数据计算了其热降解动力学参数, 从而进一步证实了三元复合物的形成以及 PAA 在三元复合物形成过程的作用。

关键词: 三元复合物; 形成; 光谱

The search for material-specific applications of natural polymers is currently a topic of great interest due to increasing environmental concerns and diminishing

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petrochemical resources^[1]. The formation of biodegradable starch-based polymers has been investigated over the past decades^[2]. Currently, starch, vegetable proteins are the main renewable resources that have been used to produce biodegradable materials due to its low price and abundant availability^[3,4]. With the excellent biocompatibility of starch and vegetable protein, starch-protein complexes have been applied in many industries including food, pharmaceutical, packaging,

cosmetic or potentially as engineering plastics^[5,6]. However, there is a continuing need to modify the properties of these materials to enhance novel application. Usually, conjugation of carbohydrates to vegetable protein directly may pose several problems because the vegetable protein tends to aggregate and reverse in solution state^[7]. It is difficult to achieve a cross-linking between starch and vegetable protein macromolecules in normal condition. Hence, it is necessary to use synthetic polymer with reactive group that is capable of reacting with the two kinds of natural polymer. One of the most promising polymers for such a purpose is polyacrylic acid (PAA). In our study, the addition of PAA could effectively induce the interaction between SPI and starch.

Fluorescence and UV-Vis absorbance spectroscopy are useful approaches for investigating intermolecular interactions. They can be used as non-destructive analytical techniques to provide information on the presence of fluorescence molecules and their environment in all sorts of biological samples^[8-10]. Resonance light scattering (RLS) measurement is a sensitive method to detect the formation of complex as well as the aggregation of biomacromolecules, which has proved that it can provide more information in the field of complex formation^[11,12].

In this article, a new approach to fabricate *starch/PAA/SPI* ternary complexes by the interactions between biopolymer (starch and SPI) and PAA was studied by fluorescence, UV-Vis and RLS spectra. In addition, in order to gain deeper insight into the complexation mechanism, the thermal stability and the kinetics of the thermal decomposition were also investigated based on RLS data.

1 Experimental

1.1 Materials and Samples Preparation

All chemicals are analytical-reagent grade or the highest available purity. All aqueous solutions were prepared with distilled, deionized water. Water-soluble starch was obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd and directly diluted in doubly distilled water with the concentration of 2.0 mg/mL. A stock solution of SPI (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd) was prepared by dissolving commercially soy protein

isolate powder in doubly distilled water with a volume concentration of 15%, and its pH was adjusted to 8.0 using 1 M NaOH solution. The dispersion was then centrifuged at 3500 r/min for 5 min, and the upper supernatant was taken for further use. A stock solution of PAA (Acros Organic Co., Ltd., $M_w=240,000$) was prepared by dissolving 0.2 g PAA with doubly distilled water in 100 mL calibrated flask. They were all stored at 0~4 °C. A buffer solution, consisting of 0.2 M phosphoric sodium, 0.1 M citric acid, was used to control the acidity of the solution.

1.2 Apparatus

The fluorescence and resonance light-scattering measurements were recorded using a RF-5301PC spectrofluorometer (Shimadzu Co.Japan). RLS measurements were performed by scanning the excitation and emission monochromators of the spectrofluorometer simultaneously from violet to visible region using quartz cuvettes (1 cm×1 cm) (Tokyo, Japan). The UV-vis absorption spectra were collected by a UV-3150 spectrophotometer (Shimadzu Corporation, Japan). The slit width was 1 nm during the measurements. The pH values were measured by using a pHs-10A digital pH meter (Xiaoshan Scientific Instruments Plant, Zhejiang, China), which calibrated with standard pH buffer solutions.

1.3 General Procedure

The pure stock solution of SPI, starch and PAA were put into a series of 10 mL calibrated flask with 2.0 mL phosphate buffer solution, and then diluted to 10.0 mL with doubly distilled water and finally stirred thoroughly. The samples of two components complex stock solution of starch/PAA, SPI/PAA and starch/SPI were prepared by pipetting the calculated volumes of different pure stock solution and mixed with each other. At last, the samples of three components mixed solution starch/SPI/PAA with the different concentration of PAA were prepared as above procedures. The resultant mixtures were subsequently incubated at room temperature for 0.5 hr. When fixed the wavelength interval ($\Delta\lambda=0$ nm) and keep the slits 1.5 nm wide, the RLS spectra was obtained by synchronous scanning in the wavelength range of 300~600 nm. The fluorescence spectra were collected using 285 nm as the excitation wavelength. The slit (ex/em) width of the measurements

was 2.5 nm /2.5 nm.

2 Results and Discussion

2.1 Two-component Blends

Generally, the change of fluorescence intensity reflects the diversification of the molecular micro-environment. For the two component systems of starch and PAA, as shown in Fig.1(a), a gradual increase in starch fluorescence intensity was observed by increasing PAA concentration, which suggested that starch transferred from the hydrophilic environment of the aqueous solution to a more hydrophobic environment^[13]. The enhanced fluorescence intensity also reflected the interaction between PAA and starch occurs due to hydrogen bonds between the carbonyl groups of PAA and the hydroxyl groups of starch^[14,15].

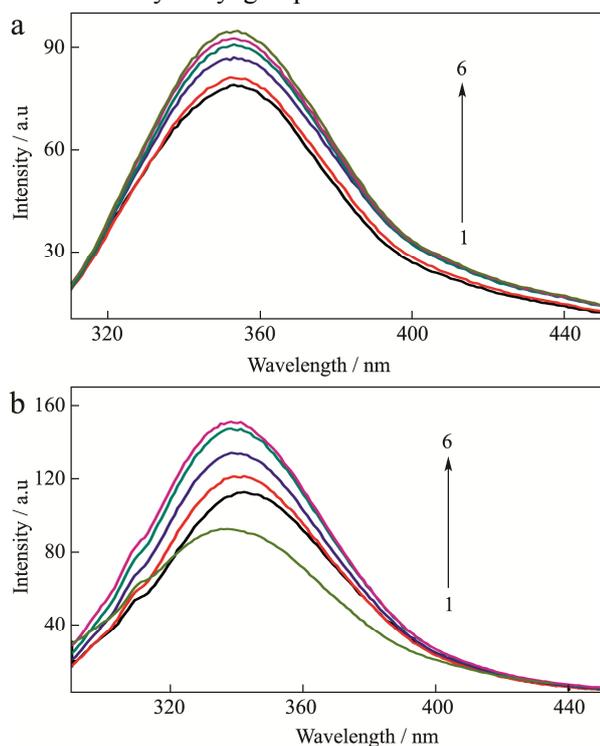


Fig. 1 Fluorescence intensity of 0.4 g/L starch (a) and SPI (b) dilute solutions as a function of PAA concentrations (from 1 to 6) (0.0, 0.5, 1.0, 1.5, 2.0, 2.5 per 10 g/L)

A different phenomenon was found when PAA and SPI were added together in solution. As shown in Fig.1(b), the characteristic fluorescence peak of SPI occurred at 340 nm. When PAA was added into the SPI solution, a specific interaction was observed by the visible enhanced and offset fluorescence signal. It can be observed that the fluorescence intensity was greatly

enhanced with the increase of PAA concentration, and λ_{max} of the solutions exhibited a red-shift (from 337 to 340 nm). It may be the multipoint electrostatic and the hydrophobic interactions between the fragments of polyelectrolytes and biomolecules that result in the formation of stable associates of PAA-SPI complexes^[16]. Furthermore, the red-shift of λ_{max} could be attributed to the increase in absorbance of the studied system by increasing PAA concentration.

From the above experiments, some synergistic interaction may occur between PAA/SPI and PAA/starch caused by the cross-linking the relevant functional groups of the two polymers. However, comparing fluorescence change produced by the cross-linked with PAA, it could be concluded that SPI has a higher reaction activity with PAA than starch. To gain an in-depth understanding of the combining information of the different two component composite, the apparent binding constant have been analyzed by Benesi-Hildebrand equation based on the fluorescence data in Fig.1(a) and Fig.1(b)^[17]:

$$1 / \Delta F = 1 / (\alpha C_B) + 1 / \{ \alpha K_f C_B [P] \} \quad (1)$$

Where ΔF is the change of SPI fluorescence intensity after the PAA addition; C_B is the total concentration of SPI; $[P]$ is the equilibrium concentration of PAA; α is the constant. Therefore, K_f can be calculated from the intercept and slope of the straight line of $1/\Delta F$ as a function of $1/[P]$ by using Eq.(1). (When $[P] \gg [B]$, $[P]$ can be replaced by C_P).

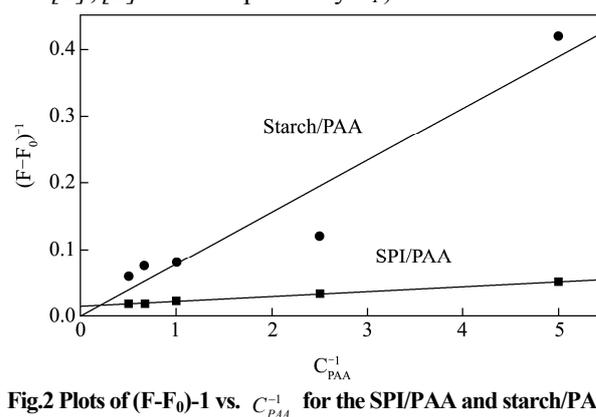


Fig.2 Plots of $(F-F_0)^{-1}$ vs. C_{PAA}^{-1} for the SPI/PAA and starch/PAA system

As can be found from Fig.2, the plots of $1/\Delta F$ versus $1/[P]$ of both two kinds of complex show a relatively good linear relationship. The binding constant was 2.02×10^5 L/mol between PAA and SPI, while 2.16×10^3 L/mol between PAA and starch. The different association

constants of starch/PAA and SPI/PAA suggested that the interaction between SPI and PAA was much stronger than the interaction between starch and PAA. It also indicated that the mutual inhibition competition may be present between SPI and starch for the combining with PAA.

2.2 Three-component Blends

On the basis of above analysis, it can be concluded that SPI can easily be combined with PAA molecules by means of e-force while the PAA can also be combined with starch shallowly to form PAA/starch complex due to hydrogen bonds. Therefore, the formation of a three-component complexes consisting of SPI, starch and PAA could be detected.

Fig.3. clearly shows the forming process of the component complex. From the fluorescence spectrum of SPI (curve *a*) and SPI/starch solution (curve *b*), it demonstrated that there was interaction between SPI and starch. When PAA was added into two components solution of SPI/starch, it not only resulted in the increase of fluorescence intensity of the study system, but also the red shift of λ_{max} from 340 to 355 nm (curve *c*). It suggested that there was association process between starch, SPI and PAA. In the SPI/starch mixing solution, the added PAA could bind with the highly branched nature starch by hydrogen bond as well as combine with SPI through electrostatic attraction.

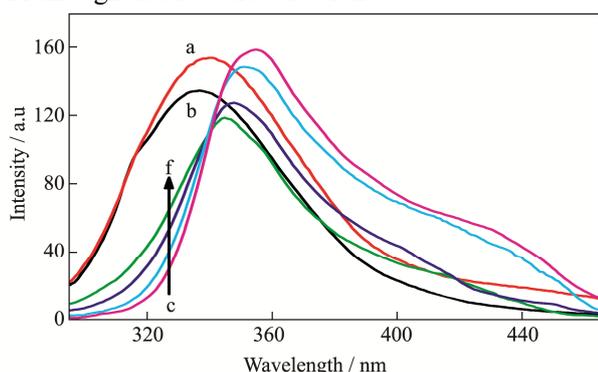


Fig.3 Fluorescence profiles of 0.2 g/L SPI (*b*) in the presence of 0.4 g/L starch solution (*a*) and PAA solution (from *c* to *f*) (0.25, 0.5, 1.0, 1.5 per 10 g/L)

Since PAA play a critical role in the novel ternary complex forming, the PAA-mediated complex formation between SPI and starch in neutral solution was investigated. From the curves (*c* to *f*) shown in Fig.3, it can be seen that with the increase of PAA concentration, the fluorescence intensity emerged durative decrease

while the λ_{max} exhibited durative blue-shift. Interestingly, the fluorescence emission spectrum at ~341 nm appeared an equal emission point, which clearly indicated the strong evidence of a new ternary complex formation consisting of PAA, SPI and starch [18].

It is also known to us that UV-Vis absorption measurement is a very simple method to explore the structural change and the complex formation. In the present study, we have recorded the UV absorption spectra of one component, two components systems and three components complex. It can be seen from the Fig.4, for the one component system, it was evident that only SPI solution shown a characteristic absorption peak appeared at about 281 nm, while PAA and starch solutions almost had no absorption. For the two components systems, only PAA/SPI had a weak absorption peak at about 280 nm, while the absorption of SPI/Starch and PAA/Starch approached zero over 250 nm. However, as the three components were mixed all together, an outstanding absorption with the strong diagnostic absorption peaks appeared at 279 nm. It indicated that the binding interaction among three components occurs. The blue shift of absorption peak from 281 to 279 nm and the increase absorption intensity could account for the changing of SPI conformation, maybe the tryptophan residues in SPI were taken into a more hydrophilic environment.

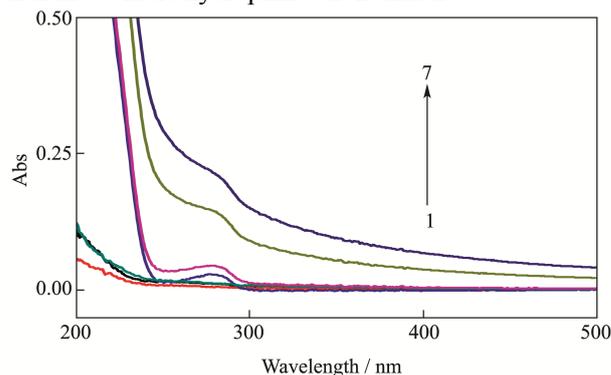


Fig.4 Absorption spectra of starch and its mixture: (1) starch (0.1 g/L); (2) PAA (0.1 g/L); (3) starch/PAA; (4) SPI (0.05 g/L); (5) PAA/SPI; (6-7) starch/SPI/PAA (concentration of PAA: 0.1, 0.2 g/L)

Another argument supporting the association of the ternary complex comes from the appearance of the rapidly enhanced RLS intensity of starch solution by the persistent addition of SPI and PAA. As shown in Fig. 5, the RLS intensity of pure starch solution was weak, but

when SPI was added into the starch solution, a specific interaction was observed by the visible enhanced RLS intensity. Since RLS intensity is dominated primarily by the particle dimension and the forming of interface in solution^[19], the result is likely due to the binding of some SPI molecules to the starch which leading to the formation of the two-component blends. At the moment, when the PAA was added into the starch/SPI solution, a drastic enhanced RLS signal of mixture system occurred. From the above phenomenon, it can be assumed that the enhancement of PAA resulted in the long range assembly of PAA on the molecular surface of SPI and starch, which induces the formation of ternary complex and results in the appear of high RLS signals under the optimal conditions.

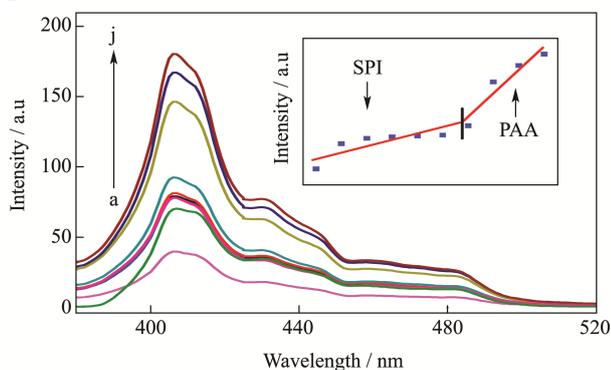


Fig.5 RLS profiles of 0.1 g/L starch solution (a) with different concentration of SPI and PAA solution. Condition: SPI (from b to g) (0.4, 0.6, 0.8, 1.0, 2.0 g/L); PAA (from h to j) (0.25, 0.5 g/L, 1.0 per10 g/L)

Fig.5 can better illustrate the association process of the three component interaction. PAA not only could induce the forming of three components complex but also taken as the third component in the ternary complex. As shown in the illustration of Fig.5, although the RLS intensity of complexes increased with the addition of SPI and PAA, the increasing trend was different when compared with each other. Apparently, the emergence of rising inflection shows some important characteristic of the interaction between these polymers. It was indicated clearly that the interaction firstly occurred between starch and SPI, and then all the three components were required to produce the ternary complexes and as the basis structural units in the complex.

Therefore, it can be inferred that the formation of ternary complex was mainly dependent on the

cross-linking action induced by PAA. Obviously, PAA played a crucial role in the formation of the new ternary complex. In short, starch, SPI and acrylic acid polymers can be generated a new ternary complex through a specific interaction between the three kinds of functional groups.

2.3 Thermal Stability of the Ternary Complex

Generally, complexes involving noncovalent bonds are reversible. In this case, investigating thermal stability of the covalent bond compounds can not only further confirm the formation of the complexes, but also obtain some useful information of the complex structure.

In the above sections, the forming mechanism of the ternary complex involved starch, SPI and PAA has been clarified clearly. On this basis, dynamic process of the formation can be inspected by investigating the temperature dependence of the intensity of RLS spectrum. Fig.6 displayed the temperature dependences of normalized RLS intensities (at the maximum scattering wavelength) of the SPI/PAA, PAA/SPI and starch/PAA/SPI solution. it can be seen that the thermal degradation temperature of starch/PAA/SPI ternary complex was higher than that of the binary complex. The different degradation stability reflected the different combination information of the binary complex and the ternary complex. Obviously, the addition of PAA induces the attacking of starch to the SPI, which increases the degradation temperature of the ternary complex. Consequently, the number of PAA can be used to modulate the degradation behavior of the ternary complex.

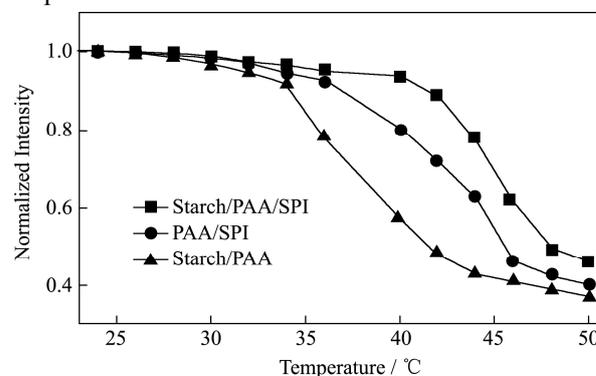


Fig.6 Relationship between temperature and the normalized RLS intensities (at the maximum scattering wavelength) of SPI/PAA, PAA/SPI, and starch/PAA/SPI solution

In order to better explain the interaction between the

three kinds of polymers, the thermal stability of the two-component complex and the ternary in solution were studied by calculating the apparent decomposition activation energy. The apparent decomposition activation energy for the thermal stability of PAA/SPI, starch/PAA and starch/PAA/SPI determined from the RLS curves could be expressed as a function of the relative extent of change for RLS intensity. It is known that the common approach for describing the thermal decomposition kinetics is the Avrami model [20]. Hereinafter, the relative extent of thermal decomposition at different temperature was calculated to perform a quantitative analysis of the dynamics of this system.

$$\frac{d(\alpha)}{dT} = \frac{A}{\Phi} e^{-E/RT} (1-\alpha) \quad (2)$$

Where $\alpha = (I_0 - I) / I_0$ is the extent of change of RLS intensity, which on behalf of the relative extent of the thermal decomposition processing. $\Phi = dT / dt$ is the heating rate, here keep a fixed constant in this case, E_a is the apparent activation energy, A is the exponential factor, and the simplified formulary can be obtained as follow.

$$\ln[\ln[1/(1-\alpha)]] = -E_a / RT + \ln[(R / E_a)(A / \Phi)T^2] \quad (3)$$

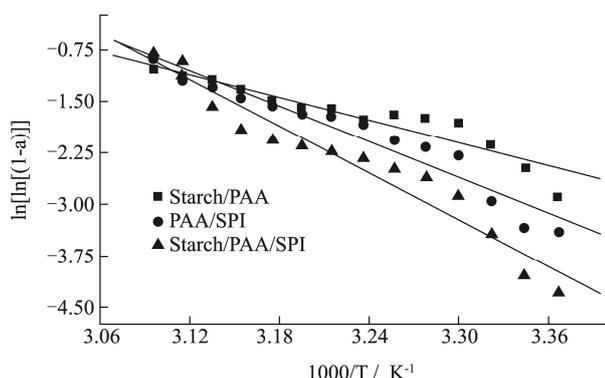


Fig.7 Kinetic studies of thermal decomposition by plotting $\ln[\ln[1/(1-\alpha)]]$ vs. 10^3T^{-1}

By using equation 3 and the experimental data in Fig.6, the apparent decomposition activation energy based on RLS files was estimated. As shown in Fig.7, both E_a of different complex can be obtained from the fitting lines of $\ln[\ln[1/(1-\alpha)]]$ versus $1/T$. The slope and calculated apparent decomposition activation energies E_a of different samples were listed in the Table 1. the E_a values for the samples of starch/PAA, SPI/PAA and

starch/PAA/SPI were 45.93, 72.17, and 94.19 kJ/mol, respectively. This suggested that the ternary complex embodied a stronger association interaction and the relatively strong thermal stability, which leads to the increase of apparent activation energies of the decomposition process [21].

Table 1 Activation energy during thermal treatment, as indicated by the RLS methods

Sample	E_a /(kJ/mol)	R^2
starch/PAA	45.93	0.939
PAA/ SPI	72.17	0.967
Starch/PAA/SPI	94.19	0.967

3 Conclusions

In this study, we have presented a novel ternary complex consisting of starch, soy protein isolate and polyacrylic acid by three-component intermolecular reactions. The data obtained from fluorescence, UV and RLS spectra provide detailed information about the structure and composition of the forming process. The hydrogen bonding between the oxygen atom of the carbonyl group of PAA and the hydrogen of the hydroxyl group of starch leads to the formation of the starch/PAA complex, and a subsequent intramolecular interaction between SPI and PAA leads to the formation of SPI/PAA complex. It is clearly indicated that PAA plays an important role to bridge between starch and protein. The thermal stability and the apparent activation energy of thermal decomposition of the binary and ternary complex in solution were also obtained by kinetics analysis based on RLS dates, which further indicated the role of PAA in the formation of the ternary complex.

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