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SHORT COMMUNICATION

Selective Fluorescent Detection of Aspartic Acid and Glutamic Acid Employing Dansyl Hydrazine Dextran Conjugate

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Abstract Highly water soluble polymer (DD) was prepared and evaluated for its fluorescence response towards various amino acids. The polymer consists of dansyl hydrazine unit conjugated into dextran template. The conjugation enhances higher water solubility of dansyl hydrazine moiety. Of screened amino acids, DD exhibited selective fluorescence quenching in the presence of aspartic acid (Asp) and glutamic acid (Glu). A plot of fluorescence intensity change of DD against the concentration of corresponding amino acids gave a good linear relationship in the range of 1×10^{-4} M to 25×10^{-3} M. This establishes DD as a potential polymeric sensor for selective sensing of Asp and Glu.

Keyword Dextran polymer · Amino acid (AA) sensor · Aspartic acid (Asp) and glutamic acid (Glu) · Dansyl hydrazine

Introduction

Over the past decade, the development of water-soluble dye conjugated polymer has found their various applications, e.g. sensing biological probe [1], chemotherapeutic agent [2], drug carrier [3], and sensor [4]. Due to the unique property of conjugated polymer such as increasing solubility and stability in an aqueous environment. This makes a conjugated polymer applicable and practical in the field of analytical research [5–8]. Recently, we have demonstrated the application of luminol conjugated dextran as a selective fluorescent probe for arginine (Arg). The tethering of luminol moiety into the dextran template does not only increase the water solubility of luminol unit, it also provides a unique conformation for selective recognition of Arg [9]. Despite the importance of techniques and methods for determination of amino acids (AA), only a handful of AA sensors have been reported [10, 11].

Of all essential amino acids, aspartic acid (Asp) and glutamic acid (Glu) play important roles in many biological and physiological pathways. Acute ischemic stroke is known with the correlation with the elevated level of Glu in blood. Glu is well known for the biosynthesis of butyric acid, an important compound in the central nervous system [12, 13]. Asp is recognized for its facilitating in the tricarboxylic acid (TCA) cycle. Research studies demonstrate the use of potassium salt of Asp as a remedy for the treatment of heart, liver diseases and diabetes [14, 15].

In this work, we reported a novel dansyl hydrazine dextran conjugate (DD) as a selective polymeric sensor for Asp and Glu. The tethering of dansyl hydrazine moiety into the polymeric dextran framework enhanced the water solubility of incorporated dansyl hydrazine units. DD was employed as a selective fluorescence polymeric sensor for amino acids. In the presence of Asp and Glu, the fluorescence intensity of DD was significantly inhibited. The proposed sensing mechanism of DD towards Asp and Glu is originated from the selective binding of either Asp or Glu to dextran. Then, the internal charge process (ICT) is inhibited, which triggers the

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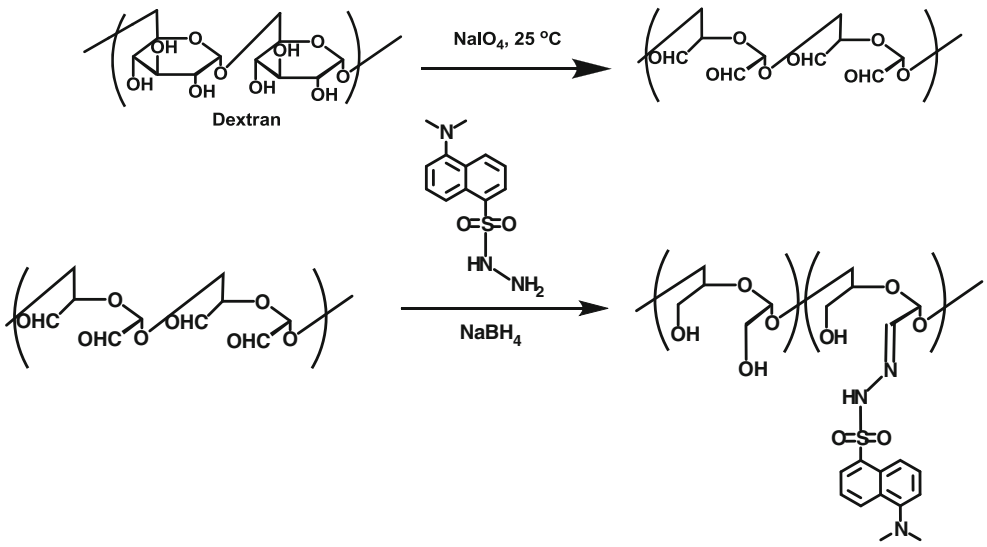
Scheme 1 Synthetic scheme of dansyl hydrazine dextran conjugate (DD)



fluorescence quenching of the nearby fluorophore, dansyl hydrazine. DD is, therefore, a good candidate for selective fluorescence sensing of Asp and Glu in an aqueous media.

Table 1 Elemental analysis and molecular weight determination of DD

Entry	%C	%H	%N	Composition of compound	MW (GPC)	PDI (M_w/M_n)
DD	52.01	6.35	8.03	$(Dan)_{525}$–$(Glc)_{1052}$	2.75×10^5 g/mol	1.34

Glc glucose unit, *Dan* dansyl hydrazine unit

Experiment

Materials

All amino acids, dextran, dansyl hydrazine and other related chemicals were purchased from Sigma Aldrich. All reagents were used without further purification.

Apparatus

Elemental analysis for the synthesized dansyl hydrazine-dextran conjugate (DD) was analyzed by National Metal

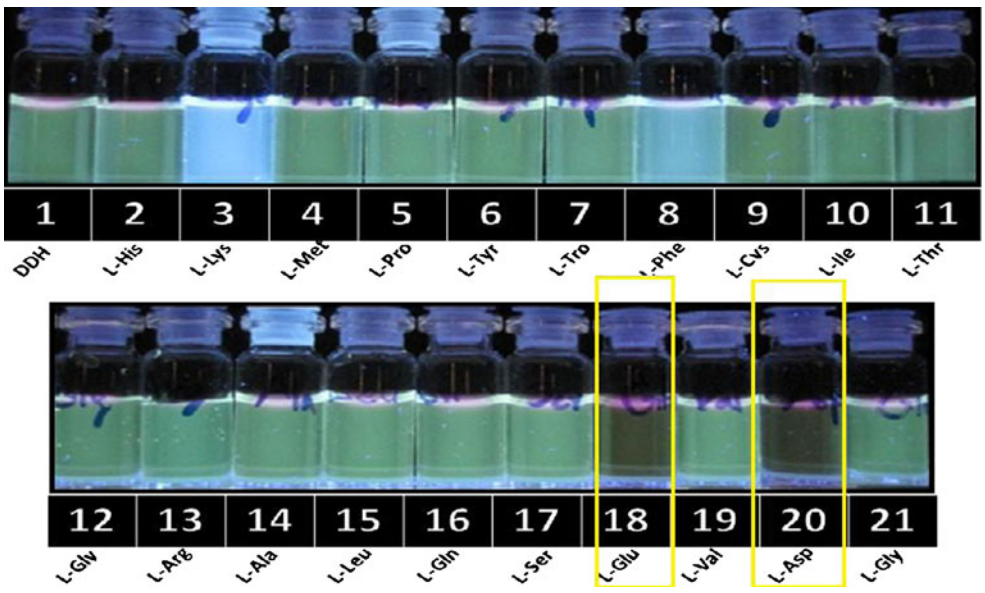


Fig. 1 Photographs of DD (5 μM) in 1%PBS buffer upon the addition of 20 amino acids, the concentration of amino acids is 25 mM. Excitation wavelength (λ_{Ex}) was 275 nm

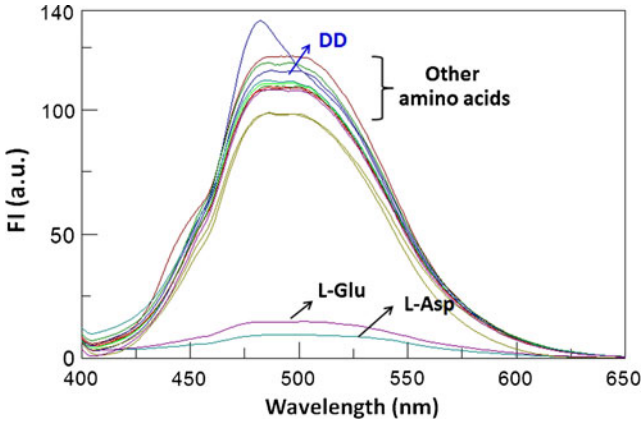


Fig. 2 The fluorescence spectra of DD (5 μM) in 1%PBS buffer upon the addition of 20 amino acids. The concentration of amino acid is 25 mM. Excitation wavelength (λ_{Ex}) was 275 nm

and Materials Technology Center (TMEC) (Thailand). Fluorescence measurements were carried out using a FP-6300 spectrofluorometer (JASCO) equipped with a xenon lamp source and a 1.0-cm quartz cell, and the scan speed was 600 nm min^{-1}.

Synthesis of Dansyl Hydrazine Dextran Conjugate (DD)

The preparation of DD was achieved according to the literature procedure with a slight modification [16]. Briefly, dextran (M_w=200 kDa) was oxidized with sodium periodate ($NaIO_4$) to form oxidized dextran. Oxidized dextran was conjugated with dansyl hydrazine via hydrazone formation. Sodium borohydride ($NaBH_4$) was used for the reduction of hydrazone and remained formyl group. DD appears as yellow powder.

Results and Discussion

Dansyl hydrazine dextran conjugate (DD) was prepared according to our previous protocol with a slight modification [16]. Briefly, dextran was oxidized by $NaIO_4$ followed by the addition of dansyl hydrazine (Scheme 1). The imine bond was reduced using sodium borohydride ($NaBH_4$) chemistry to give DD as yellow powder. To confirm the chemical composition of DD, it was submitted to an elemental analysis and the number of incorporated dansyl hydrazine units was determined and the polymer consisted of 525 dansyl hydrazine units (Table 1). The molecular weight of DD was determined by Gel Permeable Chromatography (GPC) and found to be 2.75×10^5 g/mol with the polydispersity index (M_w/M_n) of 1.34.

The selectivity of DD towards 20 amino acids was then investigated. In 1 % PBS buffer, DD, itself, showed strong fluorescence intensity (Fig. 1). In the presence of 20 amino acids, the fluorescence enhancement was observed when lysine (Lys) and phenylalanine (Phe) were added into DD solution (Fig. 1). These effects were similarly observed when Lys and Phe were added into dansyl hydrazine solution. This indicated that the fluorescence enhancement of DD solution in the presence of Lys and Phe is not originated from specific interactions between DD and those corresponding amino acids. In the presence of other amino acids, no fluorescence response was clearly observed. However, when DD solution was added with Asp and Glu, the fluorescence intensity of DD was significantly inhibited. It was clearly shown that the presence of either Asp or Glu attenuated the fluorescence signal of DD at the maximum wavelength of 475 nm which is the characteristic absorption peak of DD (Fig. 2). It was clearly observed that the DD is a selective polymeric chemosensor for Asp and Glu.

The quenching mechanism is proposed that the dextran framework acts as a binding template for either Asp or Glu. The pendant dansyl hydrazine triggers the internal charge transfer (ICT) upon the binding of corresponding amino acids. Since the ICT process highly depends on the choice of donor and acceptor. It is proposed that DD adopts a flexible conformation to suit the selective binding to either Asp or Glu. Due to the similarity in their structural frameworks, DD was unable

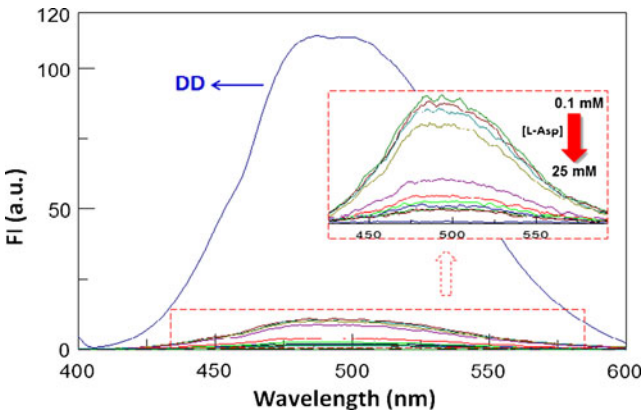


Fig. 3 Fluorescence titration of DD (5 μM) in the presence of various concentrations of Asp. Excitation wavelength (λ_{Ex}) was 275 nm

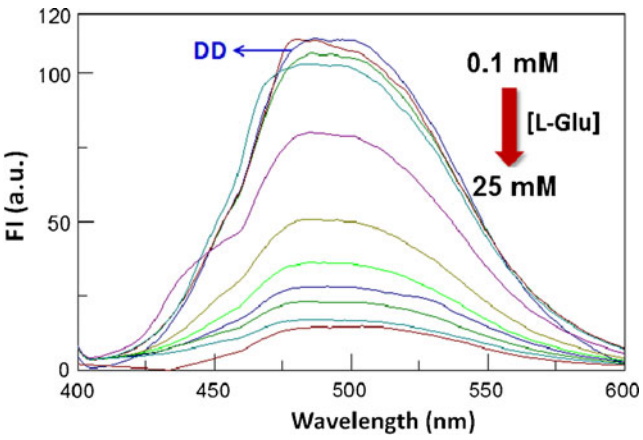


Fig. 4 Fluorescence titration of DD (5 μM) in the presence of various concentrations of Glu. Excitation wavelength (λ_{Ex}) was 275 nm

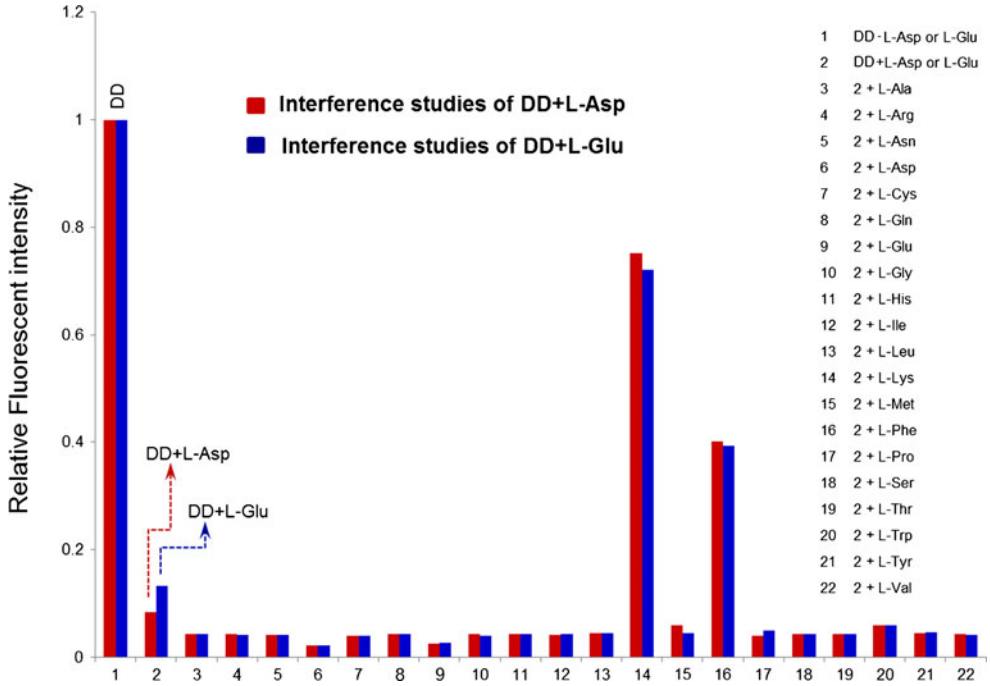


Fig. 5 The effect of fluorescence quenching of Asp and Glu in the presence of co-existing amino acids. DD solution (5 μM) contains 25 mM of Asp (*red bar*, 2) or Glu (*blue bar*, 2) and co-existing amino acids (25 mM) in 1%PBS buffer. Excitation wavelength (λ_{Ex})=275 nm

to differentiate between these two amino acids. Upon binding, the fluorescence quenching of DD in the presence of either Asp or Glu is initiated presumably due to the inhibition of ICT channel in which dansyl hydrazine and a side chain of either Asp or Glu (Asp = β-carboxyl; Glu = γ-carboxyl) are good electron donor/acceptor [17]. The proposed mechanism is similarly observed and explained by the fluorescence quenching of polymer by L-arginine. [9].

The fluorescence emission of DD was conducted at different concentrations of Asp and Glu while the concentration of DD was maintained at 5 μM. It was clearly shown in Figs. 3 and 4 that increasing concentrations of either Asp or Glu resulted in the decreasing of fluorescence intensity of DD. After the concentration of either Asp or Glu reached 25 mM, the fluorescence signal of DD was significantly inhibited (Figs. 3 and 4). Comparing to the degree of fluorescence quenching, it was found that Asp had a more pronounced effect and quenched the fluorescence intensity of DD with a greater extent compared to Glu.

Figure 5 shows the interference study for the fluorescence responses of DD in the presence of co-existing amino acids. It was shown that DD solution contained either Asp or Glu with co-existing Phe or Lys had a slightly enhanced effect for the fluorescence intensity of DD. This was expected according to previously described that Phe and Lys cause the fluorescence enhancement of dansyl hydrazine moiety. The presence of other co-existing amino acids gave no significance in the fluorescence response of DD.

It was shown that a plot of fluorescence intensity change of DD against the concentration of Asp and Glu ranging from 1×10^{-4} to 25×10^{-3} M, the fluorescence quenching of DD intensity was started to observed as low as 1×10^{-4} M of either Asp or Glu was added into DD solution. From data obtained in

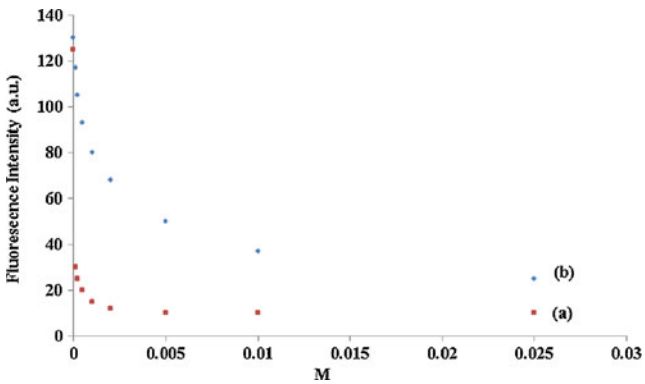


Fig. 6 A plot of fluorescence intensity change of DD in 1 % PBS buffer with various concentrations of (**a**) Asp and (**b**) Glu

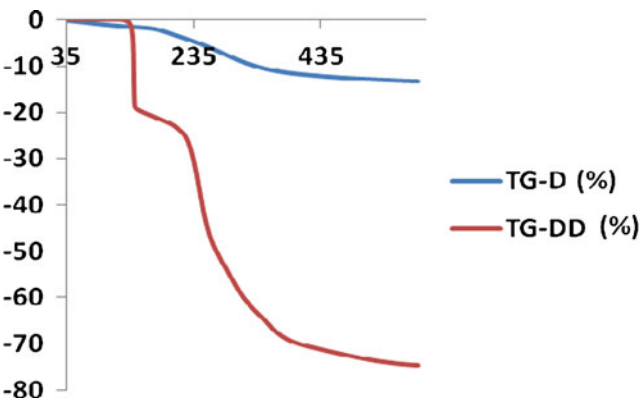


Fig. 7 TGA thermogram of D and DD

Fig. 6a and b, it was shown that DD could be used for the detection of Asp and Glu at a sub-millimolar level.

The thermogravimetric analysis (TGA) of dextran (D) and dansyl dextran conjugate (DD) were used to identify components which were degraded under nitrogen atmosphere (Fig. 7). The result was shown that the weight loss of DD was found over two regions, i.e. 0–118, 118–280 °C. These resulted from the loss of dansyl hydrazine moiety and the decomposition of dextran.

Conclusions

In summary, a novel fluorescence polymer chemosensor based on dextran polymer (DD) was prepared and evaluated for its fluorescence response towards all essential amino acids. In the presence of either Asp or Glu, the fluorescence intensity of DD was significant quenched. The fluorescence quenching mechanism of DD either by Asp or Glu is originated from the ICT process which is triggered upon the specific binding of either Asp or Glu to DD. DD was used for the detection of Asp and Glu at a sub-micromolar level.

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