Enantioselective Binding of α-Amino Acids at Poly(L-glutamic acid)-functionalized Monolayer Surfaces

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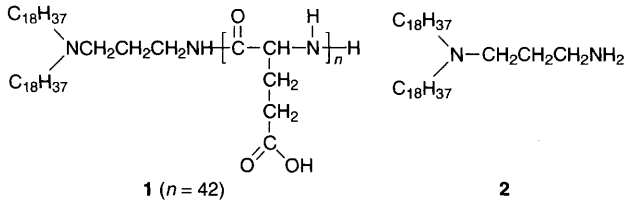
A poly(L-glutamic acid)-functionalized monolayer **1** has the ability to capture α-amino acids enantioselectively in aqueous solution due to a highly assembled structure of the α-helical poly(L-glutamic acid) segments at the monolayer surface.

We have established a well-organized amphiphilic polyelectrolyte assembly system at the air–water interface.[1] Two-dimensionally aligned polyelectrolytes have shown unique properties different from those in bulk aqueous phase: for instance poly(methacrylic acid)-based monolayers have the ability to read out the chain length of the corresponding guest polymers such as poly(ethylene glycol) as the molecular area variation of the monolayers. To model the structural feature of proteins, an amphiphilic poly(L-glutamic acid) (PLGA) **1**, which is well known to take a higher-ordered structure such as an α-helix, has also been aligned on water. The secondary structure of the PLGA segment (α-helix–β-sheet–random coil) has been readily controlled by changing the surface pressure of the monolayer (*i.e.* monolayer phase).[2] These organized PLGA assemblies are also expected to provide a site in which a guest molecule can be specifically captured. Recently, Maruyama *et al.*[3] reported enantioselective permeation of α-amino acids through thick polymer films based on α-helical PLGA having amphiphilic side chains. The authors noted an importance of the aggregation structure of PLGA in the polymer films for such an enantioselectivity. In the present study, we describe a specific binding of α-amino acid isomers at deposited one-layer LB films of PLGA-based amphiphiles **1** on quartz plates.



1 (n = 42) **2**

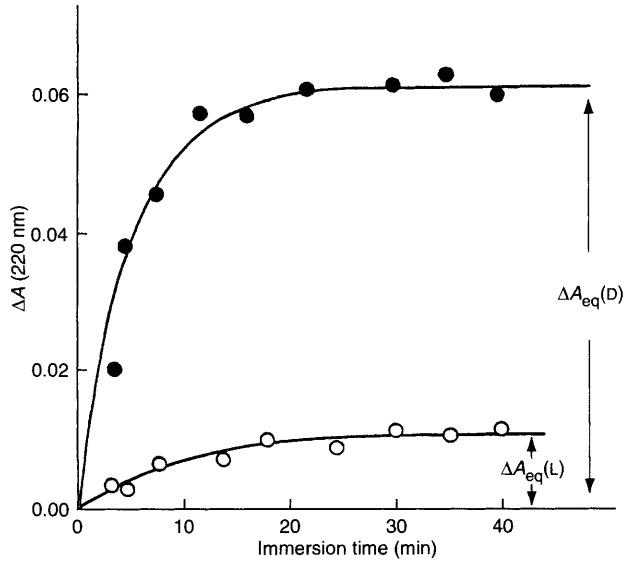


Fig. 1 Absorbance changes ΔA at λ = 220 nm for a set of **1**-monolayer covered quartz plates when separately immersed in aqueous solutions of D- (●) or L-Trp (○): [Trp] = 1 mmol dm^{-3}

The synthesis of **1** carrying the PLGA segment (number-average degree of polymerization, n = 42) was described previously.[2] Tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) were purchased commercially and used without further purification. The monolayer was obtained by spreading a benzene–DMF (7 : 3 *v/v*) solution of **1** on highly purified water (Milli-Q system, Millipore Ltd.). The concentration of the spreading solution was about 1.5 mg cm^{-3}. The surface pressure–area curve was measured at 20 °C as described previously.[4]

The PLGA amphiphile **1** was found to form a stable monolayer with a collapse pressure of 55 mN m^{-1} from the surface pressure–area isotherm on pure water (not shown here). The hydrophobic quartz plate† was lowered at a speed of 10 mm min^{-1} through the monolayer on a subphase at 30 mN m^{-1}, pH 5.7, at which the monolayer is in a well-condensed phase, and a one-layer LB film, in which the PLGA segments of **1** are exposed to water phase, was deposited on each side of the quartz plate. The transfer ratio was close to unity. A CD (circular dichroism) spectrum of this LB film‡ exhibited two negative bands at 222 and 208 nm,[5,6] indicating the formation of an α-helical conformation.

Subsequently, these monolayer-covered surfaces were subjected to binding experiments with α-amino acids. The binding processes were followed as an absorbance difference (ΔA) in UV spectra before and after binding of α-amino acids. Fig. 1 shows the absorbance changes of ΔA at 220 nm for a set of **1**-monolayer-covered quartz plates when they were separately immersed in aqueous solution of L- or D-Trp (1 mmol dm^{-3})

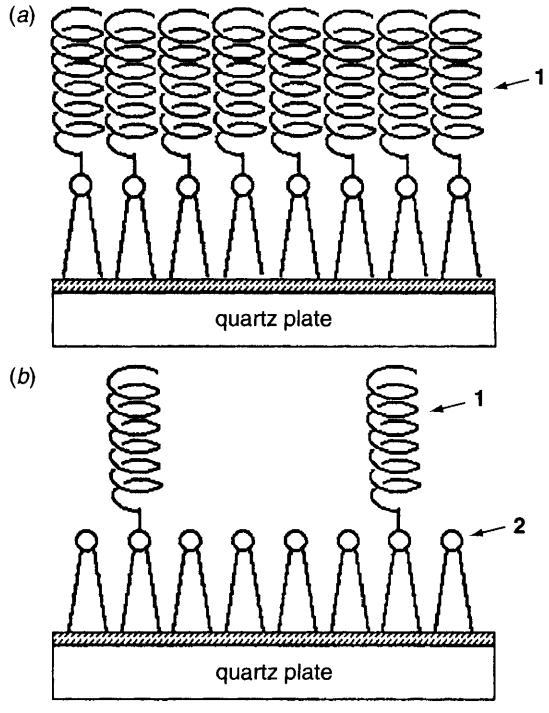


Fig. 2 Schematic illustration for monolayers from (*a*) pure **1** and (*b*) a mixture of **1** and **2** (molar fraction of **1**, 0.2)

(λ_{max} 220 nm).§ The values of ΔA for both isomers are observed to increase gradually upon immersion due to binding of Trp at the monolayer surfaces, and then levelled off within *ca.* 30 min. The amount of surface-bound D-Trp to the **1** layer at equilibration [ΔA_{eq}(D)] is found to be much larger by a factor of *ca.* 6 than that of L-Trp [ΔA_{eq}(L)].

To examine the universality in such a selectivity observed for Trp, the same experiment was carried out for two other α-amino acids, Phe and Tyr. The selectivity between D- and L-isomers estimated as a ratio of ΔA_{eq}(D)/ΔA_{eq}(L) was 8 and 3 for Phe and Tyr, respectively. It is clear that the organized PLGA surface of the **1** monolayer has the ability to discriminate α-amino acids enantioselectively although the degree of discrimination varies for the amino acids studied, probably due to differences in steric effects, hydrophobicity and/or other factors of the α-substituent of the α-amino acids.

To confirm if the observed enantioselectivity derives from an ordered-aggregation structure of the PLGA segments on the monolayer surface, a mixed monolayer, composed of **1** and PLGA segment-free **2**, was transferred onto the quartz plate in the same way and was subjected to the adsorption experiment with Trp. The component molecules of **1** and **2** are assumed to be homogeneously dispersed within the monolayer since the molar fraction of **1** in the mixed monolayer was adjusted to be 0.2. As a result, this mixed monolayer is expected to capture both D- and L-Trp (though values of ΔA_{eq} are *ca.* 0.001, much less than those of pure **1** monolayer) but not to provide any significant selectivity between D- and L-isomers. Therefore, it is evident that an assembled structure of the PLGA segment [Fig. 2(*a*)] plays a key role in causing enantioselectivity of α-amino acids.

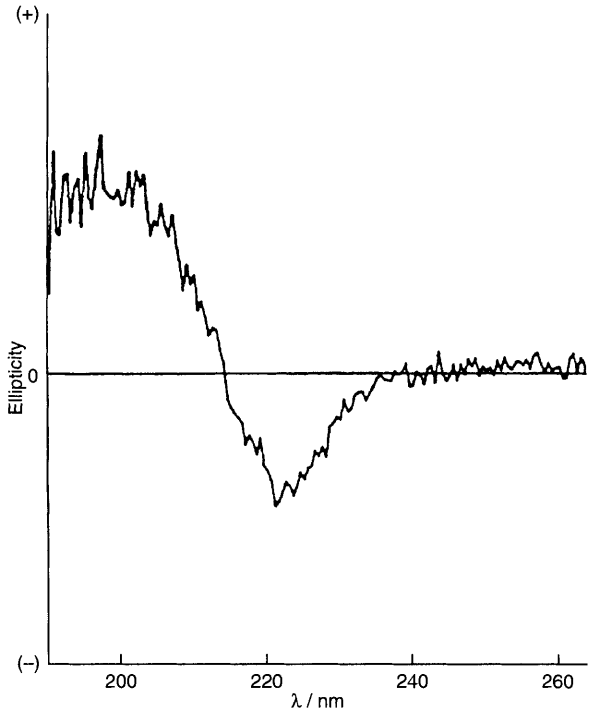


Fig. 3 Differential CD spectrum of the **1**-monolayer covered quartz plates before and after adsorption of a racemic mixture of DL-Trp

Finally, we examined selective binding of a racemic mixture of DL-Trp at the **1** monolayer surface under the same experimental conditions as those described in Fig. 1. Equilibration of adsorption occurred after *ca.* 30 min on the basis of measuring ΔA as described above. Fig. 3 displays a differential CD spectrum before and after adsorption (60 min) of DL-Trp at the **1**-monolayer-covered quartz plate. The spectrum gives a typical CD pattern of D-Trp. This result undoubtedly demonstrates that the D-isomer was preferentially adsorbed by the **1** monolayer, though minor adsorption of the L-isomer can not be excluded. A more quantitative estimation for adsorption from racemic mixtures will be studied further using a variety of techniques.

In conclusion, the present study demonstrates that the D- and L-isomers of α-amino acids are captured enantioselectively by a PLGA assembled monolayer, in which a highly assembled structure of the α-helical PLGA segments plays an important role in causing such a selectivity. This polymer assembly system is of particular significance not only in view of its applicability for a biomolecular sensor but also in view of a receptor model of biomembrane surfaces.

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Footnotes

† All quartz plates used in this study were hydrophobically coated with a thin layer (*ca.* 100 Å) of poly(dimethylsiloxane) prior to use.

‡ The CD spectrum of the LB film was measured by putting the quartz plate to which the one-layer film was attached in to a quartz cell (pathlength 10 mm) filled with pure water.

§ The same experiment was also performed on the monolayer-free (bare) quartz plate, and no significant adsorption of Trp was observed.

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