Induced Circular Dichroism of Benzyl Chromophores Bound to Helical Polypeptides

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Extrinsic optical activity was observed for symmetric side chain chromophores in helical polypeptides. Poly-β-benzyl-L-aspartate, poly-β-*p*-nitrobenzyl-L-aspartate and poly-γ-*p*-nitrobenzyl-L-glutamate gave induced circular dichroism in the wavelength regions corresponding to the absorption bands of the benzyl and the *p*-nitrobenzyl chromophores of the side chains in their dilute solutions. Moreover, poly-γ-benzyl-D-glutamate gave induced circular dichroism due to the benzyl chromophore in its concentrated solutions which formed a liquid crystalline phase. The extrinsic optical activity thus observed was explained as the consequence of the loss of the rotational freedom of the chromophores relative to the α-helical structure and to the cholesteric helical structure of the polypeptides.

Blout and Stryer[1] reported that extrinsic optical activity is induced in the wavelength region corresponding to the absorption band of acridine orange (AO) or acriflavine when the dye molecule becomes bound to the α-helical poly-α,L-glutamic acid (PLGA) molecule or to nucleic acids. Recently, we made more intimate investigation into the optical activity of AO bound to the carboxylate group of the side chain of PLGA in the neutral and the alkaline pH regions.[2,3]

On the other hand, it has been found that poly-γ-(1-naphthylmethyl)-L-glutamate[4] and poly-γ-(β-*N*-carbazolylethyl)-D-glutamate[5] also exhibit some circular dichroism (CD) bands in the wavelength regions corresponding to the absorption bands of their side chain chromophores when the polypeptides are dissolved in helix solvents at low concentrations, such as 10^{-3} mol of the peptide residue/l.

Loucheux and Duflot[6] reported that the nitrobenzyl chromophore in α-helical PLGA exhibits a CD band at 295 nm which they assigned to the n-π^* transition of the nitrobenzyl group. They offered for the origin of the extrinsic optical activity of the symmetric chromophore of the side chain in the α-heical polypeptide the following three mechanisms, (i) strong chromophore-chromophore interactions; in this case, the α-helix served only to hold constant the relative geometry between neighboring chromophores, (ii) local interactions between the chromophore and the dissymmetric field of the α-helix and (iii) the perturbation to the chromophore by the dissymmetric field of the chiral carbon in the peptide residue.

In our recent investigation[7] concerning the optical activity of D-phenylglycine in comparison with that of L-phenylalanine, we indicated that the perturbation by the dissymmetric field of the chiral carbon to the transition of the phenyl group in an asymmetric molecule decreases abruptly as the rotational freedom of the phenyl chromophore is increased by increase in the chiral carbon to phenyl chromophore distance, and that the perturbation is expected to be only trivial for the chromophore separated by more than four atoms from the chiral carbon atom. The perturbation by the chiral carbon to which the side chain is attached should therefore have a very small contribution to the induced circular dichroism of the benzyl chromophores bound to the α-helical polypeptides studied in this paper, unless limitation of the rotation of the chromophore is caused by some origin.

In the present paper, the extrinsic optical activity of the benzyl chromophores bound to the helical polypeptides is examined and this optical activity is correlated to the rotational freedom of the chromophore.

Experimental

Materials. Poly-β-benzyl-L-aspartate (PBLA) and poly-γ-benzyl-D-glutamate (PBDG) were prepared by the conventional polymerization of the *N*-carboxyanhydrides of the parent amino acids. Unless otherwise noted, the degrees of polymerization of these polymers were 170 and 1320, respectively. Poly-β-*p*-nitrobenzyl-L-aspartate (PNO_2BLA) and poly-γ-*p*-nitrobenzyl-L-glutamate (PNO_2BLG) were prepared by the method reported by Ledger and Stewart.[8] Ethylene chlorohydrin and tetrachloroethane were purified by ordinary methods. The other solvents were spectral grade ones and were used without further purification.

Methods. The infrared (IR) spectra were measured by a Hitachi EPI-S_2 infrared spectrometer. The cell for observation consisted of two NaCl plates separated by a 30 μm lead spacer, and the polymer solution was inserted between the plates. The absorption spectra, the CD spectra and the optical rotatory dispersion (ORD) curves were observed for the concentrated polymer solution held between two quartz plates spaced by a suitable aluminum foil. In the case of the dilute solution, a usual optical quartz cell was used. The absorption spectra were recorded on a Hitachi EPS-3T spectrometer. The CD and the ORD measurements were carried out by a JASCO J-20A dichrograph and a J-15 spectropolarimeter, respectively.

Results and Discussion

Extrinsic Optical Activity of Benzyl and p-*Nitrobenzyl Chromophores Bound to Helical Polypeptides in Dilute Solutions.* Poly-γ-*p*-nitrobenzyl-L-glutamate shows a definite negative CD band in the neighborhood of 275 nm. The CD spectrum is shown in Fig. 1. In spite of the existence of the anomalous dispersion around 275 nm in its ORD curve, we can obtain Moffitt's b_0 of −540 from the fairly linear Moffitt plot in the longer

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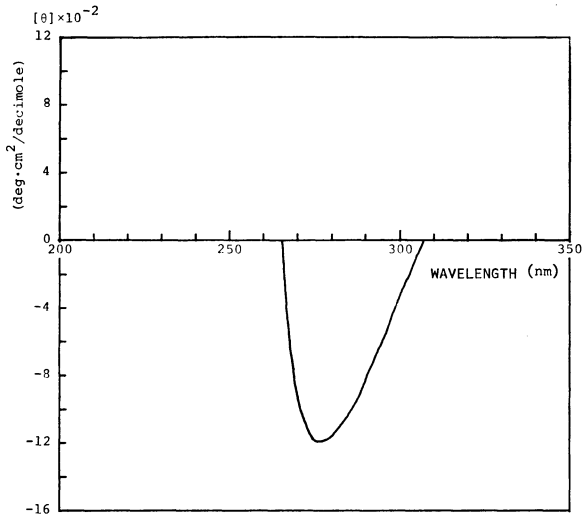

Fig. 1. CD spectrum of PNO_2BLG in *N,N*-dimethylformamide ($\Delta\varepsilon/\varepsilon = -3.8\times10^{-5}$ at 275 nm).

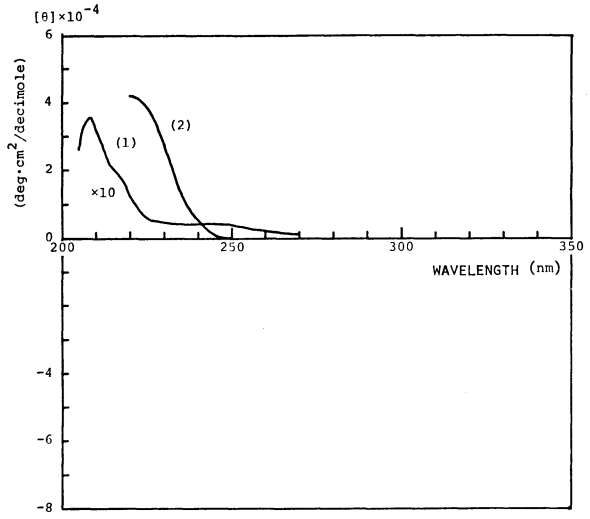

Fig. 2. CD spectra of (1) NO_2BLG in water and (2) PBDG (DP=165) in ethylene chlorohydrin; "×10" denotes that the spectrum is magnified 10 times longitudinally.

wavelength region than 390 nm indicating the right-handed α-helical structure. As is shown in Fig. 2, we cannot detect any CD band in the wavelength region longer than 260 nm for γ-*p*-nitrobenzyl-L-glutamate and PBDG at low concentrations, such as 10^{-3} mol of glutamyl residue/l.

From these, it is deduced that the introduction of the nitro group into the side chain benzyl group of the polyglutamate intensifies the interaction between side chain groups leading to the increase of the probability of their definite orientation relative to the polypeptide main chain. This unexpected loss of the rotational freedom of the side chain chromophore causes interaction between the transition dipole moment of the nitrobenzyl chromophore and that in the α-helix main chain, and is the very reason why circular dichroism appears in the absorption region of the nitrobenzyl chromophore despite the distance between the chromophore and the polypeptide main chain.

Goodman *et al.*[9-11] reported that the nitrobenzyl group in PNO_2BLA forms a rigid side chain helix around the α-helix of the polypeptide main chain, but that the nitrobenzyl group in PNO_2BLG is not in a dissymmetric field and can rotate freely. Our above-mentioned observation of the circular dichroism or the anomalous optical rotatory dispersion ascribed to the aromatic chromophore is attained by the development of the J-20A dichrograph with a low noise-high output amplifier. Loucheux and Duflot reported that the CD spectrum of the copolymer of L-glutamic acid with *p*-nitrobenzyl-L-glutamate shows a positive peak at 296 nm in an aqueous solution at pH 4.5.[6] It is interesting that the incorporation of small amount of the γ-*p*-nitrobenzyl-L-glutamate unit into poly-L-glutamic acid results in a circular dichroism which differs both in the sign and the wavelength of the maximum from those in the homopolymer of γ-*p*-nitrobenzyl-L-glutamate.

The dilute solution of poly-β-benzyl-L-aspartate in chloroform or dichloromethane gives a weak but undoubted CD band centered at 252 nm, as is shown in Fig. 3. A large positive CD band appearing at 222 nm, means that PBLA exists in the left-handed α-helical structure.[12,13] We also observed the optical rotatory dispersion of the same chloroform solution of PBLA as is shown in Fig. 4 in comparison with that of the left-handed α-helical structure of PBDG. The feature is very similar to that reported by Blout and coworkers,[13] and shows an anomalous dispersion giving a negative Cotton effect in the absorption region of the benzyl chromophore. The extrinsic optical activity observed here is due, in similar manner to the case of PNO_2BLG, to the definite orientation of the benzyl chromophore relative to the main chain α-helix, which is brought about by the interaction between benzyl residues of PBLA whereas we have not detected any circular dichroism for the benzyl group of β-benzyl-L-aspartate. More crowded arrangement of the benzyl group in PBLA around the main chain α-helix because of the lack of one methylene group in the side chain leads to the increase in the interaction between benzyl

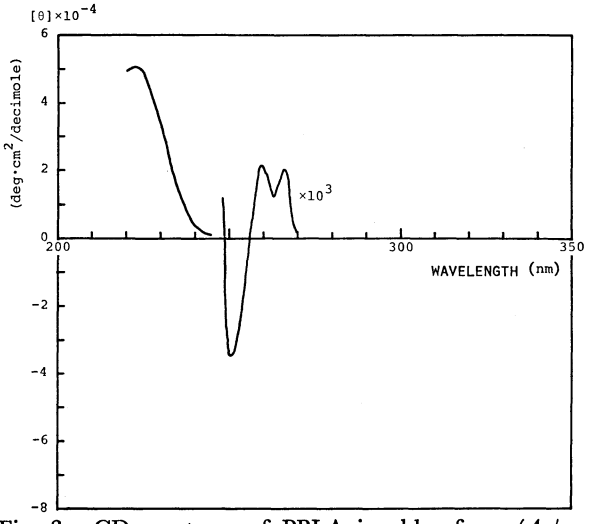

Fig. 3. CD spectrum of PBLA in chloroform ($\Delta\varepsilon/\varepsilon = -6.2\times10^{-5}$ at 252 nm); "×10^3" denotes that the spectrum is magnified 10^3 times longitudinally.

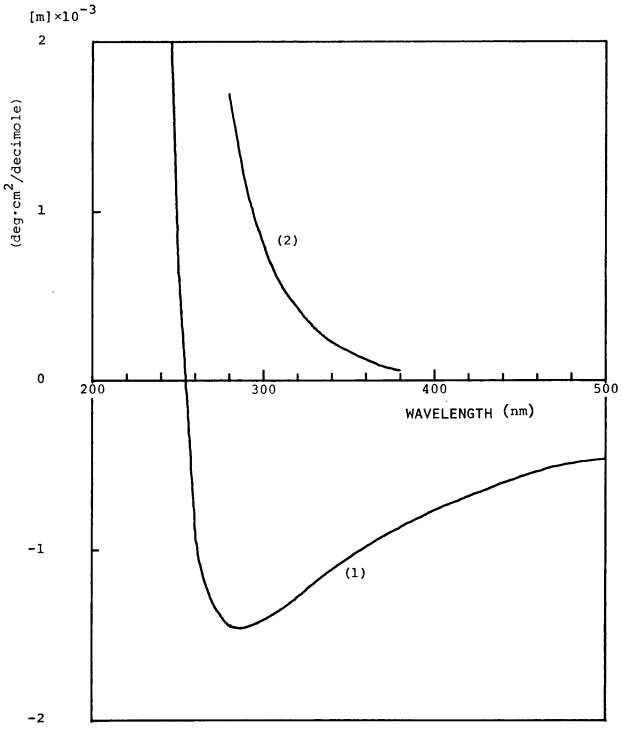

Fig. 4. ORD curves of (1) PBLA in chloroform and (2) PBDG in dichloromethane (0.018 mole of glutamyl residue/l).

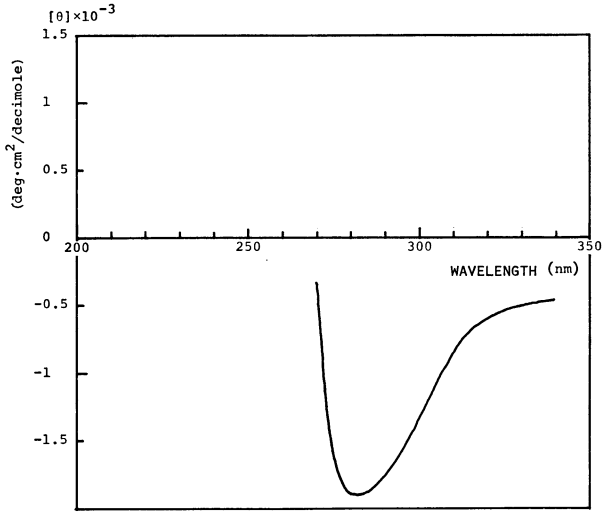

Fig. 5. CD spectrum of PNO_2BLA in *N,N*-dimethylformamide ($\Delta\varepsilon/\varepsilon = -6.9\times10^{-5}$ at 280 nm).

chromophores of PBLA, giving rise to the circular dichroism detected in PBLA while such is not the case for PBDG.

In Fig. 5 the CD spectrum of PNO_2BLA is shown. No circular dichroism is detectable in the wavelength region longer than 250 nm for *β-p*-nitrobenzyl-L-aspartate. The CD band of PNO_2BLA around 290 nm is also ascribed to its benzyl chromophore. The α-helical structure of PNO_2BLA in *N,N*-dimethylformamide has been ascertained by Goodman *et al.*[9] The molar ellipticity $[\theta]_{280}$ of PNO_2BLA is much larger than $[\theta]_{275}$ of PNO_2BLG. This again reflects the fact that the side chain in the polyaspartate is constrained in its orientation as a result of their shorter side chain length than that of the polyglutamate by one methylene group. It is remarkable that the absolute value of the optical anisotropy factor $\Delta\varepsilon/\varepsilon$ is only slightly larger in PNO_2BLA than in PBLA. The α-helical sense of PBLA is opposite to that of PNO_2BLA, so this difference in $\Delta\varepsilon/\varepsilon$ cannot be correlated in a straightforward way to the difference in the magnitude of the interaction between side chain aromatic chromophores.

It must be noticed that the size of the interaction between side chain chromophores of the above-mentioned polypeptides is no more than necessary to align the chromophores around the definite orientation relative to the main chain helices of the polypeptides and is not so strong as to cause strong chromophore-chromophore interactions mentioned in mechanism (i) by Loucheux and Duflot resulting in the Davydov splitting of the CD band.

Induced Circular Dichroism of Benzyl Chromophores Bound to Helical Polypeptides in Concentrated Solutions.

PBLG in α-helical conformation forms a cholesteric liquid crystal in its concentrated solutions (10—50 wt% of the polymer).[14] A chirality is caused by the existence of a unidirectional twist on the array of a set of equispaced parallel planes in which α-helical axes lie, nevertheless the solution forms an isotropic domain as a whole because of the random orientation of the axes of the cholesteric helix in the macroscopic domain. The extrinsic optical activity due to the side chain benzyl group is expected to occur in such a concentrated PBL(D)G solution since a regularly ordered array of the side chain chromophore may exist in such a phase.

In the concentration range of PBDG less than 0.1 mol of glutamyl residue/l in dichloromethane, no evidence of the occurrence of the higher order structure can be detected. ORD curves for such a concentration range (Fig. 4) show monotonic patterns as is encountered for the usual polypeptide solution having the α-helical structure. There is no Cotton effect observed in the wavelength region longer than 250 nm, and we get reasonable b_0 values around +650 from the good linearity of the Moffitt plot in the range of 280 nm—350 nm.

When the concentration is raised over 0.4 mol of glutamyl residue/l, it is found from the microscopic examination that the cholesteric liquid crystalline phase is formed in the system. For this system, the ORD curve, giving an anomalous dispersion near 255 nm, is quite different from those for less concentrated systems (Fig. 6), and it cannot be decided from this ORD curve whether or not the α-helical structure is retained since the Moffitt plot no longer gives a straight line and does not give a definite b_0. On the other hand, IR mesurements indicate that the α-helical structure is retained up to the concentration of 0.75 mol of glutamyl residue/l since the IR bands (amide I and amide II bands) which are ascribed to the α-helical structure appear at 1650 cm^{-1} and at 1550 cm^{-1} and a band at 1690 cm^{-1} due to the non-hydrogen bonded carbonyl group is absent.[15]

As expected from the remarkable concentration

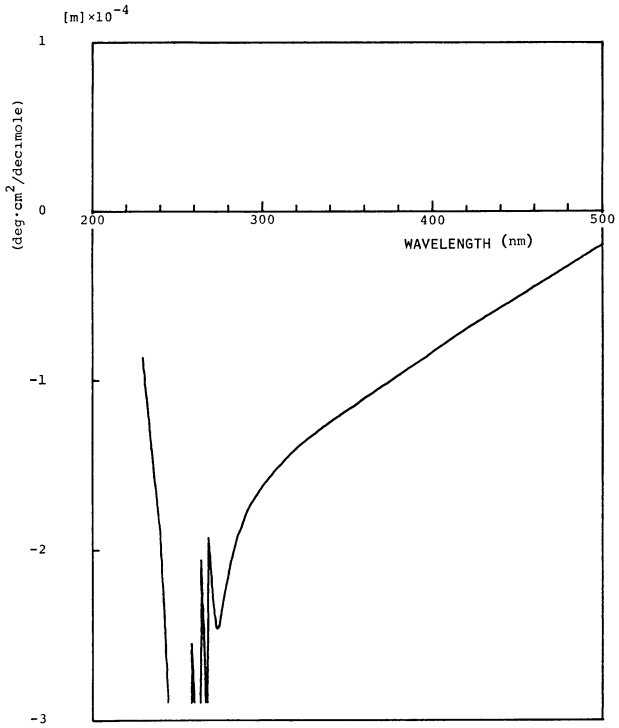

Fig. 6. ORD curve of concentrated PBDG solution in dichloromethane (0.74 mol of glutamyl residue/l).

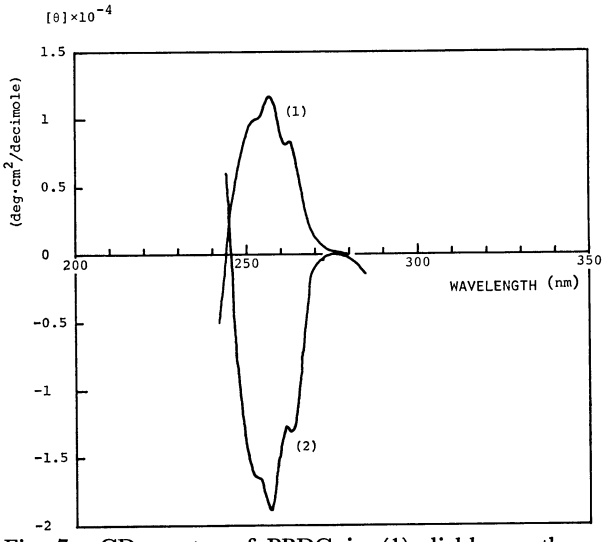

Fig. 7. CD spectra of PBDG in (1) dichloromethane (0.48 mol of glutamyl residue/l, $\Delta\varepsilon/\varepsilon = +0.014$ at 256 nm) and (2) tetrachloroethane (0.46 mol of glutamyl residue/l, $\Delta\varepsilon/\varepsilon = -0.022$ at 256 nm).

dependence of the ORD curve, a CD band near 255 nm really appears at higher concentrations than 0.4 mol of glutamyl residue/l in dichloromethane. The cholesteric phase is also formed when PBDG is dissolved in other helix solvents such as dichloroethane and tetrachloroethane, giving similar CD bands near 255 nm, but the signs of the CD bands in these solvents are opposite to that in dichloromethane. The observed CD spectra are shown in Fig. 7. On the other hand, it has been confirmed by the polarizing microscopic observation that the nematic liquid crystalline phase of PBDG is formed when this polypeptide is dissolved in the solvent mixture of dichloromethane and dioxane (4: 1 in volume). No CD band around 255 nm appears in this nematic phase.

Extremely condensed phase is realized locally around closed-packed α-helices lying in parallel equispaced planes making up the cholesteric helix, and thus the benzyl chromophore attached to the α-helix is imposed to take a definite arrangement relative to this plane. This, in turn, means that the side chain benzyl chromophore assumes a definite orientation relative to the cholesteric helix. We can deduce, through the analogous way to that in the case of the dilute solution of PNO_2-BLG, that the extrinsic optical activity induced to the aromatic chromophore originates from the interaction between the aromatic chromophore of the polypeptide and the "helix." The solvent dependence of the sign of the Cotton effect of the benzyl chromophore of PBDG in the cholesteric liquid crystalline phase can probably be explained by the solvent dependency of the sense of the cholesteric helix reported by Robinson.[14]

No extrinsic optical activity can be observed for PBDG existing in the nematic liquid crystalline phase, while the benzyl chromophore of PBDG in such a phase can be regarded as fixed in its orientation relative to the α-helix in the closed-packed structure as well as in the cholesteric phase and an extrinsic optical activity is expected to appear due to the dissymmetric field of the α-helical structure. To explain this fact, it might be necessary to assume that there exist enantiomeric arrangements of the benzyl chromophore subsisting simultaneously in the closed-packed plane of the nematic structure. This probably prevents the closed-packed plane of the nematic structure from arranging in a helical sense to form a cholesteric helical structure.

Again, as in the dilute solution of the polypeptide, there exists no Davydov pair of CD bands here, and so the benzyl chromophore of PBDG in the cholesteric liquid crystalline phase is not so closely stacked as to produce the interaction between electric transition moments in benzyl chromophores.

The optical anisotropy factor $\Delta\varepsilon/\varepsilon$ is 1.4×10^{-2} for this case, and this value is surprisingly large compared to those for PBLA, PNO_2BLA and PNO_2BLG in the dilute solutions which are -6.2×10^{-5}, -6.9×10^{-5}, and -3.8×10^{-5}, respectively. This fact is indicative that the dissymmetric field for the first case cannot be sought in the same origin as for the rest. This also supports the idea that the extrinsic optical activity of PBDG in its concentrated solutions should not be attributed to the α-helical structure of the polypeptide, but to the helical structure of the cholesteric liquid crystalline phase.

Conclusion

The dissymmetric field for the present case comes from two origins, namely the α-helical structure of the polypeptide and the cholesteric helical structure in the liquid crystalline phase of the polypeptide. The extrinsic optical activity appears when the benzyl chromophore loses its freedom of rotation to be oriented more rigidly relative to this dissymmetric field. PBLA has one less methylene group in its side chain than PBDG, realizing

more crowded arrangement of the benzyl group around the main chain α-helix. This leads to the definite orientation of the benzyl chromophore relative to the α-helix, giving rise to the extrinsic optical activity in the dilute solution of PBLA. The introduction of the nitro group into the benzyl chromophore increases the bulkiness of the side chain and the magnitude of the electrostatic interaction between side chains. This increase results in the enhancement of the probability of a definte orientation of the benzyl chromophore of PNO_2BLG relative to the α-helix of PNO_2BLG in its dilute solution or in the inversion of the left-handed α-helical sence of PBLA to the right-handed one of PNO_2BLA in the dilute solution. The benzyl chromophore of PBDG in its concentrated solution forming the cholesteric liquid crystalline phase takes a definite orientation in the closed-packed structure in the parallel equispaced planes making up the cholesteric helix.

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