May, 1989] © *1989 The Chemical Society of Japan* NOTES *Bull. Chem. Soc. Jpn.*, *62*, *1671—1673 (1989)* 1671

Solubilization of β-Carotene in Water by Water-Soluble Linear Macromolecules

Isamu Inamura,* Masayuki Isshiki, and Takeo Araki
Department of Chemistry, Faculty of Science, Shimane University, Matsue 690

(Received September 21, 1988)

Synopsis. β-Carotene was solubilized in water by the water-soluble linear macromolecules of poly(*N*-vinylpyrrolidone) (PVP), polyethylene glycol (PEG), poly(vinyl alcohol) (PVA), and dextran. Absorption of β-carotene in aqueous macromolecular solutions were red-shifted. The rate of photobleaching of β-carotene in water depended on the polymer species.

It is well-known that carotenoids are soluble in oragnic solvents but insoluble in water. Therefore, these have been studied in vivo and in organic solvents by many investigators,[1,2] but not in water. However, Shibata et al.[3] recently solubilized β-carotene in water by forming complexes with egg albumin. As shown in this report, we have succeeded in solubilizing β-carotene in water by means of water-soluble linear polymers of PVP, PEG, PVA and dextran.

Experimental

Materials. β-Carotene manufactured by Kanto Chemical Co., Inc. was used without further purification. PVP: molecular weight (mol wt), 40000 and PEG: mol wt, 20000 were purchased from Kishida Chemical Co., Ltd. PVA; PVA-117: mol wt, 77000, degree of saponification, 98.5 mol%, was kindly donated by Kuraray Co., Ltd. Dextran: mol wt, 75000 was purchased from Wako Pure Chemical Industries, Ltd. PVP, PEG, PVA, and dextran were purified by reprecipitation of them in solvent–nonsolvent, namely in water–acetone, benzene–petroleum ether, water–methanol, and water–ethanol, respectively. Bovine serum albumin (BSA) manufactured by Armour Pharmaceutical Company was used without further purification.

Method for Solubilization of β-Carotene. β-Carotene was solubilized in water as follows. An organic solvent containing β-carotene was added to a polymer/org. solv. or water solution. (The compositions of raw materials are shown in Table 1 for all the samples). The solution was evaporated to a dry β-carotene/polymer film under a reduced pressure at room temperature. A small amount of water was added onto the film, and stirred gently at room temperature until a homogeneous paste was formed. The paste was diluted with water to a given concentration of β-carotene. In the same way, β-carotene was solubilized in water by BSA or Triton X-100.

Analytical Methods. Absorption spectra at room temperature were measured with a double beam spectrophotometer, UVIDEC-510 (Japan Spectroscopic Co., Ltd.). Photobleaching experiments of β-carotene in different states were done under while light (2.7×10^{2} J m^{-2} s^{-1}) from a projector lamp.

Results and Discussion

Figure 1 shows the absorption spectra of β-carotene in different states. β-Carotene in acetone had three ab-

Table 1. Composition of Raw Materials and Visible Light Absorption Maxima of β-Carotene in Aqueous Macromolecular Solutions

Sample No.	Composition of raw materials			Absorption maxima[d)] (nm)				
	Polymer–Solvent (g) (ml)	Car[a)]–Solvent (mg) (ml)	Poly/Car[b)]					
1	PEG 0.15–benzene 5	Car 4–benzene 5	1	412	439	459	490	535
2	PEG 0.15–benzene 5	Car 4–benzene 5	1	(432)[c)]	460	494	540	
3	PEG 0.15–benzene 5	Car 4–benzene 5	1	416	439	(466)	(500)	
4	PEG 0.44–benzene 5	Car 4–benzene 5	3	(427)	452	483	524	
5	PEG 0.11–benzene 5	Car 0.5–benzene 5	6	(422)	448	477	518	
6	PEG 0.89–benzene 10	Car 4–benzene 5	6	(423)	454	478	(515)	
7	PEG 1.18–benzene 10	Car 0.5–benzene 5	64	(425)	443	470	513	
8	PEG 1.18–benzene 10	Car 0.5–benzene 5	64	(425)	440	(470)	512	
9	PVP 0.30–chloroform 5	Car 4–chloroform 5	1	(412)	(437)	460	494	543
10	PVP 0.30–chloroform 5	Car 4–chloroform 5	1	(416)	440	(465)	(500)	
11	PVP 0.60–chloroform 5	Car 4–chloroform 5	2	(424)	443	(473)	514	
12	PVP 1.48–chloroform 10	Car 4–chloroform 5	5	(418)	434	(467)	(501)	
13	PVP 0.19–chloroform 5	Car 0.5–chloroform 5	5	(418)	435	(461)	(498)	
14	PVP 1.18–chloroform 10	Car 0.5–chloroform 5	32	(425)	441	(470)	510	
15	PVP 1.18–chloroform 10	Car 0.5–chloroform 5	32	(420)	439	(470)	(510)	
16	PVP 1.18–chloroform 10	Car 0.5–chloroform 5	32	(418)	436	(460)	(500)	
17	PVA 1.5–water 10	Car 0.5–acetone 5	21	(416)	450	478	(515)	
18	PVA 1.5–water 10	Car 0.5–acetone 10	21	(414)	438	462	(495)	
19	dextran 1.5–water 10	Car 0.5–acetone 5	22	410	437	(467)	(502)	
20	dextran 1.5–water 10	Car 0.5–acetone 5	22	412	434	(460)	(495)	
21	BSA 0.62–water 9	Car 0.5–ethanol 1	10	419	(437)	(467)	(508)	
22	BSA 0.62–water 9	Car 0.5–ethanol 1	10	410	(434)	(462)	(502)	
23	Triton X-100 1–acetone 5	Car 0.5–acetone 5		(410)	(436)	460	486	

a) β-Carotene. b) Mole ratio polymer/β-catotene. c) The number in parenthesis indicates an absorption maximum of a shoulder. d) Concentrations of β-carotene are 2×10^{-5} mol dm^{-3}.

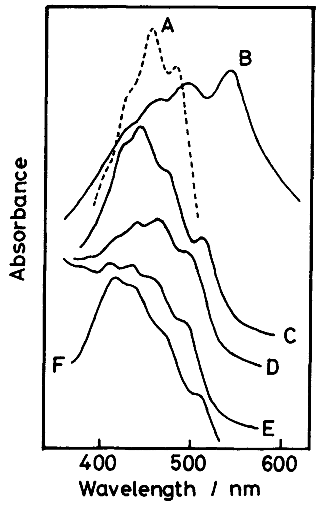

Fig. 1. Absorption spectra of β-carotene in different states. Concentrations of β-carotene are 2×10^{-5} mol dm^{-3}. The sample numbers indicated in Table 1 are shown in parentheses. (A) β-carotene in acetone, (B) β-carotene in aqueous PEG solution (No. 2), (C) β-carotene in aqueous PVP solution (No. 14), (D) β-carotene in aqueous PVA solution (No. 18), (E) β-carotene in aqueous dextran solution (No. 20), (F) β-carotene in aqueous BSA solution (No. 21).

sorption bands at 430, 450, and 481 nm. On the other hand, β-carotene in aqueous solutions of the water-soluble linear polymers and BSA had a red-shifted absorption band at the wavelength longer than 495 nm, in addition to the other three bands of shorter wavelength.

Also in Table 1, there was observed a red-shifted absorption band at the wavelength longer than 495 nm, in addition to the three or four bands of shorter wavelength, for all the β-carotene in aqueous macromolecular solutions. The red-shifted absorption appeared in the 495—543 nm range with different samples. The mole ratio polymer/β-carotene of raw material did not affect the wavelength of red-shifted absorption. It is noted, however, that the absorption bands of longer wavelength than 535 nm were observed only for the solutions with the mole ratio polymer/β-carotene of 1:1. Further, it was ascertained that the absorption spectra of β-carotene in aqueous PVP and PEG solutions were almost identical with those of the β-carotene/PVP and PEG films described in the Experimental section, respectively (data not shown).

Figure 2 shows the time-dependent spectral change of β-carotene in aqueous PVP solution caused by irradiation of white light. The intensity of red-shifted peak at 510 nm decreased at the same rate as the other bands of shorter wavelength. Such a spectral change was observed for all the β-carotene in aqueous macromolecular solutions shown in Fig. 3, suggesting that the red-shifted bands have the same origin as the other bands of shorter wavelength.

Figure 3 shows the results of photobleaching of β-carotene in different states by irradiation of while light. The rate of photobleaching of β-carotene in aqueous macromolecular solutions depended on the polymer species; the order of the rate of photobleaching was dextran>PVA>BSA>PEG>PVP. β-Carotene in aqueous dextran solution was photobleached almost completely after 10 min irradiation, while β-carotene in aqueous PVP solution remained unphotobleached by 35% after 360 min irradiation.

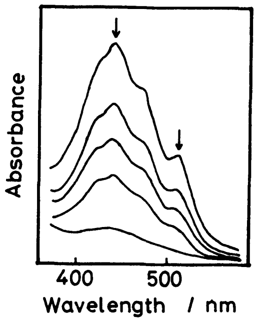

Fig. 2. Time-dependent spectral change of β-carotene in aqueous PVP solution (No. 14) caused by irradiation of white light. (Time=0, 60, 120, 360 and 900 min.)

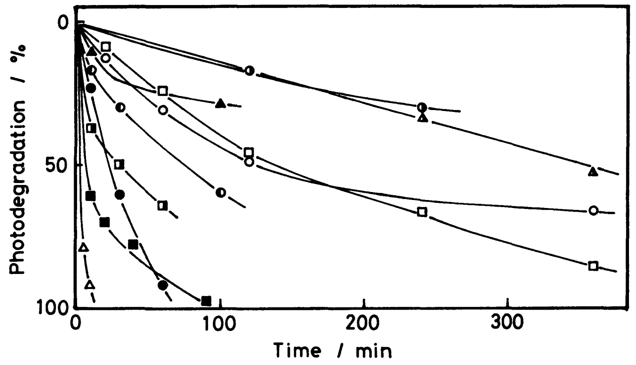

Fig. 3. Photobleaching of β-carotene in different states by irradiation of white light. The wavelengths monitoring the photobleaching were those of absorption peaks in the 440—465 nm range. The initial concentrations of β-carotene were 2×10^{-5} mol dm^{-3}. Δ, β-carotene in acetone; ◑, β-carotene in hexane; ▲, β-carotene in benzene; ◨, β-carotene in aqueous acetone (80%) solution; ◐, β-carotene in aqueous Triton X-100 solution (No. 23); ○, β-carotene in aqueous PVP solution (No. 14); □, β-carotene in aqueous PEG solution (No. 8); ■, β-carotene in aqueous PVA solution (No. 17); △, β-carotene in aqueous dextran solution (No. 19); ●, β-carotene in aqueous BSA solution (No. 22).

It should be also noted in Fig. 3 that β-carotene in acetone, hexane and benzene was more stable against light irradiation than that in aqueous macromolecular solutions. This result is unexpected because chlorophyll in the macromolecular complexes in water was more stable against light irradiation than chlorophyll in organic solvents.[4] Since β-carotene in aqueous acetone (80%) solution, having three absorption bands at 432, 457, and 483 nm, was photobleached at a higher rate than that in acetone as shown in Fig. 3, it is considered that the medium of water or organic solvents was an important factor in the stabilization of β-carotene under light irradiation, in addition to the presence of polymer.

Recently, Wloch et al.[5–7] investigated a long-wavelength absorbing form (LWAF) of β-carotene and believed that it represents a fine-crystalline form of the pigment where head-to-tail aggregation occurs. According to them, it can be inferred that the linear macromolecules formed complexes with the fine-crystalline form of β-carotene, resulting in solubilization of the pigment in water. The polymers probably bound it within random coil of the chain. The difference in position of the red-shifted absorption bands may reflect the variation in size of the crystals of β-carotene.

References

1) D. Siefermann-Harms, *Physiol. Plantarum,* **69**, 561 (1987).

2) B. H. Davies, "Chemistry and Biochemistry of Plant Pigments," ed by T. W. Goodwin, Academic Press, London (1976), Vol. 2, p. 38.

3) H. Shibata, C. Kurosaki, T. Kawashima, and H. Ochiai, *Agric. Biol. Chem.*, **51**, 3261 (1987).

4) I. Inamura, T. Araki, M. Shei, T. Nishikawa, H. Shibata, and H. Ochiai, *Biochim. Biophys. Acta,* **932**, 335 (1988).

5) S. Wieckowski, E. Wloch, and B. Broniowska, "Advances in Photosynthesis Research," ed by C. Sybesma, Martinus Nijhoff/Dr W. Junk Publishers, The Hague/Boston/Lancaster (1984), Vol. 2, p. 1.

6) E. Wloch and S. Wieckowski, *Bull. Pol. Acad. Sci.:Biol. Sci.*, **34**, 263 (1986).

7) E. Wloch, S. Wieckowski, and A. M. Turek, *Photosynthetica,* **21**, 2 (1987).