Electronic Spectra and Photochemistry of Coordinated Astaxanthin

Horst Kunkely and Arnd Vogler

Institut für Anorganische Chemie, Universität Regensburg, 93040 Regensburg, Germany

Reprint requests to Prof. Dr. A. Vogler. E-mail: Arnd.Vogler@chemie.uni-regensburg.de

Astaxanthin (AXT) is a natural carotenoid which provides colorations to numerous living species such as salmon. Owing to its chelate function deprotonated astaxanthin (axt$^-$) should be easily attached to metal ions. The complex Rh^{III}(phpy)$_2$(axt) with phpy$^-$ = deprotonated 2-phenylpyridine was prepared, and its electronic spectra and photochemistry were examined and compared to that of free AXT. Although different in detail, the spectra and photochemistry of AXT and Rh(phpy)$_2$(axt) show common features such as fluorescence and photoisomerization of the axt$^-$ ligand in ethanol as solvent. In CH$_2$Cl$_2$ free AXT as well as the complex undergo photooxidation owing to the reducing property of astaxanthin as an antioxidant.

Key words: Electronic Spectra, Luminescence, Photochemistry, Rhodium Complexes, Astaxanthin, Carotenoid

Introduction

Astaxanthin (AXT, Fig. 1) is a carotenoid (CAR) [1, 2] which occurs naturally and leads to the coloration of numerous living species such as salmon, krill, shrimp, and birds (*e. g.* feathers of flamingo) [3]. It provides different colors, in particular yellow to red colorations dependent on the environment of the AXT molecules. It can be isolated from natural and artificial sources. ATX is applied to generate its color in certain cases. For example, meat from salmon of aquacultures is normally rather pale but acquires its desired orange coloration when ATX is added to the nutrition [3].

Generally, the color of CARs such as vitamin A is attributed to the π-$\pi*$ transitions of the chain of conjugated double bonds [4]. Several types of environmental influences can generate spectral shifts. Steric restrictions and, in particular, charge effects are very important. Many studies have been devoted to rhodopsin [5], but it seems to be clear that the π-$\pi*$ transitions of other CARs such as AXT are modified by analogous
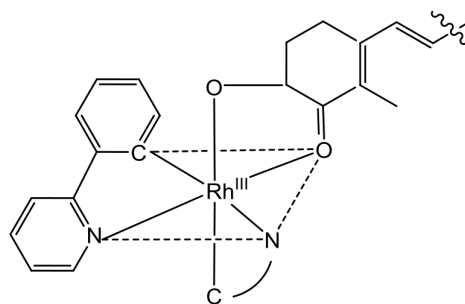
Fig. 2.

effects. With regard to charge effects, metal ions may play an important role. AXT provides a chelate function which should facilitate the formation of metal complexes. While this possibility has been recognized, well-defined metal complexes have not yet been isolated [6]. We explored this possibility and selected the complex Rh^{III}(phpy$^-$)$_2$(axt$^-$) with phpy$^-$ = deprotonated 2-phenylpyridine and axt$^-$ = deprotonated AXT for the present study (Fig. 2).

CARs are characterized by various properties. They act, for example, as antioxidants in food. Of special
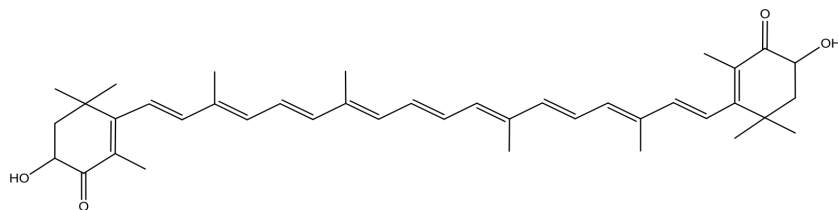
Fig. 1.

interest is the photochemistry [7, 8]. CARs including vitamin A [4] and rhodopsin [5] undergo *trans/cis* photoisomerization in analogy to other polyolefins with conjugated double bonds. Moreover, as reducing species (antioxidants) they can be photooxidized. Finally, many CARs show a fluorescence [8, 9] which is an important probe for *the excited state processes*. In the present study such properties of AXT have been examined and compared to those of AXT in the complex Rh(phpy)$_2$(axt$^-$) in order to evaluate the effect of coordination which might also occur in natural environments. Metal salts are ubiquitous compounds with important roles which are frequently not well understood.

Results

[Rh(phpy)$_2$Cl]$_2$ and astaxanthin (AXT) were commercially available. Rh(phpy)$_2$(axt) with the axt anion (= deprotonated AXT) was prepared in analogy to other complexes of the type Rh(phpy)$_2$(chelate) [10, 11]. The structure of Fig. 2 is an arbitrary choice. Other stereo-isomers of the chelate are possible and probably present. Moreover, the polyolefin chain of the axt ligand may also occur in different *trans/cis* isomers (see below). The presence of such mixtures interferes with a reliable characterization by other analytical methods. In particular, the product Rh(phpy)$_2$axt could not be obtained in crystalline state preventing a structural identification. This lack is certainly also based on the presence of different isomers. The absorption spectrum of AXT has been reported to display a pronounced band with a
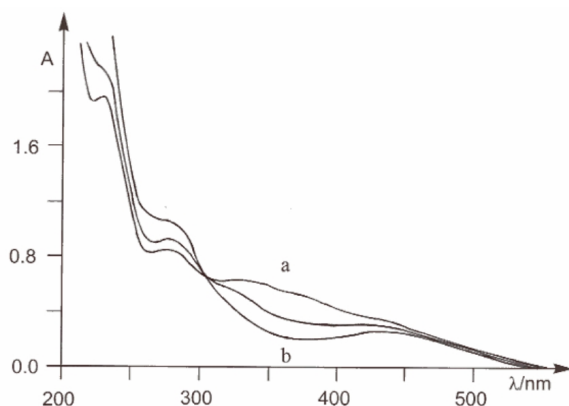

Fig. 3. Spectral changes during the photolysis of 5.4×10^{-6} M AXT in ethanol at r. t. after 0 (a), 5, and 30 (b) min irradiation time with $\lambda_{\mathrm{irr}} > 340$ nm, 1 cm cell.
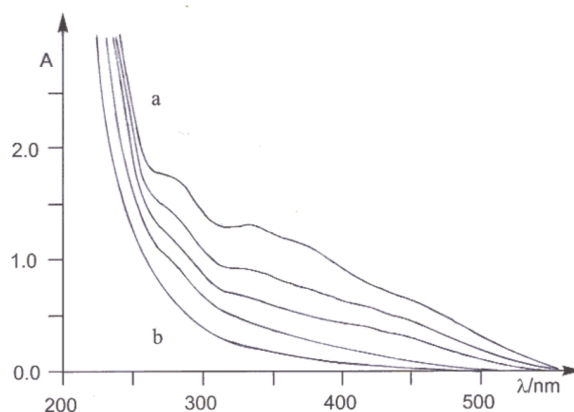

Fig. 4. Spectral changes during the photolysis of 5.4×10^{-6} M AXT in CH$_2$Cl$_2$ at r. t. after 0 (a), 2, 5, 10, and 60 (b) min irradiation time with $\lambda_{\mathrm{irr}} > 280$ nm, 1 cm cell.

maximum between 450 and 500 nm dependent on the solvent [12 – 14]. Our sample shows several absorption features as shoulders between 300 and 500 nm (Figs. 3 and 4). It is assumed that it contains different *trans/cis* isomers. Upon irradiation of this solution in ethanol the shoulders at shorter wavelength between 300 and 400 nm disappear, and only a long-wavelength feature is preserved and shows up as a new maximum at $\lambda = 440$ nm (Fig. 3). Prior to irradiation, AXT exhibits a fluorescence at $\lambda_{\mathrm{max}} = 450$ nm ($\lambda_{\mathrm{exc}} = 300$ nm). Light absorption by the new long-wavelength band of the photolyzed solution leads to a fluorescence at $\lambda_{\mathrm{max}} = 580$ nm ($\lambda_{\mathrm{exc}} = 480$ nm). Such a fluorescence of AXT has been reported before [9].

Solutions of AXT in CH$_2$Cl$_2$ show a different behavior. The photolysis leads to a bleaching of the solution. The concomitant spectral changes (Fig. 4) indicate simply a loss of AXT without the formation of a new species with a well-defined absorption spectrum. At the irradiating wavelength ($\lambda > 280$ nm) the light is only absorbed by AXT but not by the solvent. The complex Rh(phpy)$_2$(axt) is slightly soluble in a variety of solvents. The absorption spectrum (Fig. 5) is hardly solvent-dependent and shows maxima at 384 ($\varepsilon = 4500$ mol^{-1} cm^{-1}), 302 (14200), 260 (31600) and 245 nm (42000) in EtOH. The complex is light-sensitive in solution. The photolysis is accompanied by spectral changes (Fig. 5) which include rather sharp isosbestic points at 376, 330, 296, 274, and 240 nm. A new long-wavelength band appears as a shoulder at ~ 360 nm.

The complex Rh(phpy)$_2$(axt) is slightly soluble in a variety of solvents. The absorption spectrum (Fig. 5)

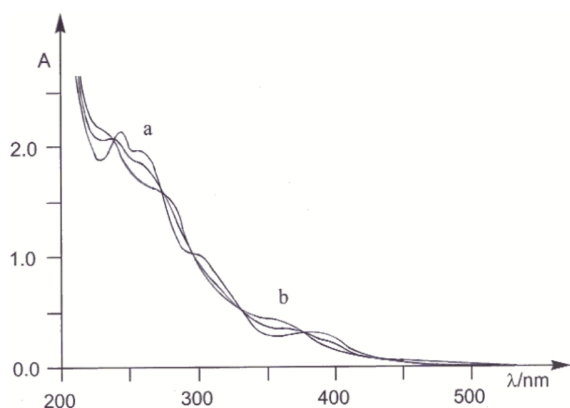Fig. 5. Spectral changes during the photolysis of 5.04×10^{-5} M Rh(phpy)$_2$(axt) in EtOH at r.t. after 0 (a), 40, and 80 (b) min irradiation time with λ_{irr} = 254 nm, 1 cm cell.

is hardly solvent-dependent and shows maxima at 384 (ε = 4500 mol^{-1} cm^{-1}), 302 (14200), 260 (31600) and 245 nm (42000) in EtOH. The complex is light-sensitive in solution. The photolysis is accompanied by spectral changes (Fig. 5) which include rather sharp isosbestic points at 376, 330, 296, 274, and 240 nm. A new long-wavelength band appears as a shoulder at ~ 360 nm.

Upon standing in the dark for 14 h the original spectrum of Rh(phpy)$_2$(axt) is almost completely recovered. While the complex shows a fluorescence at λ_{max} = 530 nm (λ_{exc} = 400 nm), light absorption by the new band of the photolyzed solution (λ_{exc} = 360 nm) is accompanied by a fluorescence at λ_{max} = 518 nm.

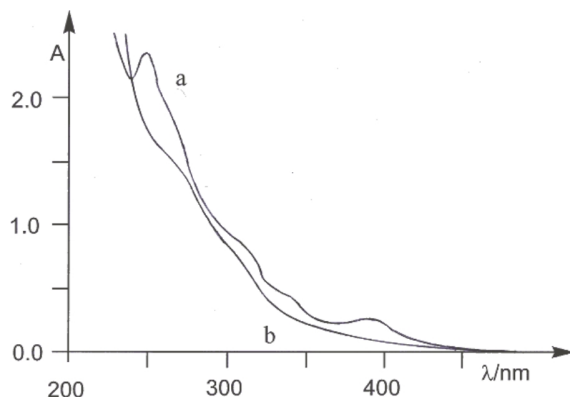When the photolysis is carried out in CH$_2$Cl$_2$ as the solvent the complex simply decays irreversibly as in-

Fig. 6. Spectral changes during the photolysis of 5.25×10^{-5} M Rh(phpy)$_2$(axt) in CH$_2$Cl$_2$ at r.t. after 0 (a) and 180 (b) min irradiation time with λ_{irr} = 254 nm, 1 cm cell.

dicated by the bleaching (Fig. 6) in analogy to the photolysis of AXT in CH$_2$Cl$_2$.

Discussion

AXT as received from a commercial source is offered as a nutritional additive for the aquatic fauna in an aquarium. This form of AXT does not display the characteristic absorption with a distinct maximum between 450 and 500 nm [12 – 14]. Additional absorption features appear at shorter wavelengths, in particular between 300 and 400 nm. They are attributed to *cis*-isomers which are known to absorb at shorter wavelength than AXT as the all-*trans*-isomer. Upon irradiation in EtOH these shorter-wavelength bands disappear and a single long-wavelength band near λ_{max} = 450 nm remains (Fig. 1). It is suggested to belong to the AXT *trans*-isomer. This assumption is supported by the occurrence of a fluorescence at λ_{max} = 580 nm [9]. Photoisomerizations of CARs are initiated by π-$\pi*$ excitation [4, 8]. They can take place in both directions, *trans* $\rightarrow$ *cis* as well as *cis* $\rightarrow$ *trans*, leading to a photostationary state. This depends on a variety of parameters such as irradiating wavelength, quantum yield and the nature of the solvent. In our case the *trans*-isomer of AXT is apparently the dominating photolysis product.

In chlorinated alkanes such as CHCl$_3$ or CH$_2$Cl$_2$ the photochemical behavior of AXT is quite different. As a well-known antioxidant it is reducing and undergoes a photooxidation in these solvents [15, 16]. Generally, this is recognized by a bleaching of the solution indicating the disappearance of the π-$\pi*$ system of the conjugated double bonds. This has been previously reported and is confirmed in our present work (Fig. 4).

The complex Rh(phpy)$_2$(axt) was synthesized in analogy to other complexes of the type Rh(phpy)$_2$(chelate$^-$) which contain a chelating O,O-anion such as tropolonate or flavonolate [10]. Since in the complex fragment Rh^{III}(phpy)$_2^+$ no low-energy CT excited states are available the attachment of an additional ligand may lead to the occurrence of new IL (intra ligand) excited states at low energies. Unfortunately, such an IL state of axt$^-$ is not apparent in the spectrum of Rh(phpy)$_2$(axt). The spectrum of this complex (Figs. 5 and 6) resembles that of [Rh^{III}(phpy)$_2$Cl]$_2$ [17] which is characterized by lowest-energy phpy$^-$ IL states. However, a careful comparison of the extinction coefficients shows that

additional long-wavelength absorptions with lower intensity are hidden in the spectrum of Rh(phpy)$_2$(axt). Moreover, while [Rh^{III}(phpy)$_2$Cl]$_2$ is not luminescent at r. t. [17], the axt complex shows a luminescence at λ_{max} = 530 nm. We suggest that this is a fluorescence which originates from the axt$^-$ ligand. In analogy to the complex Rh(phpy)$_2$(flavonolate) [10], a pronounced heavy-atom effect leading to fluorescence quenching in the axt$^-$ complex is not seen. However, the population of the axt$^-$ IL state is not only indicated by the appearance of the IL fluorescence, but also by the photochemical behavior of the complex. In this case the irradiation apparently facilitates a *trans → cis* isomerization of the axt$^-$ ligand as indicated by the blue shift of the long-wavelength absorption. Moreover, such a blue shift is also observed for the fluorescence from 530 to 518 nm. It is not clear why the *trans → cis* photoisomerization is favored in the case of the complex, but it is well known that various influences can play a significant role in the photo-isomerization of CARs. In particular, charge effects which are certainly important for the interaction with metal ions could dominate the excited state reactivity of CARs [5]. Nevertheless, the photoreaction is thermally reversed indicating the stability of the original isomer.

In chlorinated alkanes as solvents the complex Rh(phpy)$_2$(axt) undergoes a photooxidation in analogy to free AXT. The anti-oxidizing behavior of AXT is obviously preserved by coordination.

In conclusion, we have prepared a rhodium(III) complex which contains deprotonated astaxanthin axt$^-$ as a ligand. This complex, Rh(phpy)$_2$(axt), undergoes a photolysis which is attributed to *trans/cis* photoisomerization of the axt$^-$ ligand. This observation may be important with regard to the photoreactivity of astaxanthin in general since it may be also attached to other metals which are present in aquatic environments.

Experimental Section

All solvents for spectroscopic measurements were of spectrograde quality. [Rh(phpy)$_2$Cl]$_2$ and astaxanthin (AXT) were commercially available from Aldrich and www.fischfut terhandel.de, respectively, and used without further purification.

Absorption spectra were measured with a Varian Cary 50 spectrophotometer. Emission spectra were recorded on a Hitachi 850 spectrofluorometer equipped with a Hamamatsu 928 photomultiplier for measurements up to 900 nm. The luminescence spectra were corrected for monochromator and photomultiplier efficiency variations.

Rh(phpy)$_2$(axt) with axt$^-$ anion = deprotonated AXT

To a solution of astaxanthin (1.5 g, 2.6 mmol) in 100 mL ethanol (97 %) were added Na$_2$CO$_3$ (0.2 g, 2 mmol) and [Rh^{III}(phpy)$_2$Cl]$_2$ (0.5 g, 0.6 mmol). This mixture was stirred for 12 h at 60 °C. In the resulting yellow solution a grey precipitate was formed and separated by filtration (0.45 μ). The filtrate was evaporated under reduced pressure. Diethyl ether (100 mL) was added to the yellow residue. The mixture was stirred for 30 min and yellow impurities removed by filtration. The solvent of the filtrate was removed by evaporation leaving a yellow product. A sample for analysis was obtained after several recrystallizations from EtOH; yield 5.5 %. – Analysis (%) for Rh(phpy)$_2$(axt): calcd. C 73.94, H 6.71, N 2.78; found C 73.53, H 6.60, N 2.61.

[1] O. Straub, *Key to Carotenoids,* Birkhäuser Verlag, Basel **1987**.

[2] G. Britton (Ed.), *Pure and Applied Chemistry*, Vol. 63, Issue 1, **1991**; (Proceedings of the Ninth International Symposium on Carotenoids, Kyoto, Japan, **1990**).

[3] T. L. Dawson, *Color. Technol.* **2006**, *122*, 61 – 73.

[4] R. S. H. Liu, A. E. Asato, *Tetrahedron* **1984**, *40*, 1931 – 1969.

[5] S. Sekharan, M. Sugihara, V. Buss, *Angew. Chem.* **2007**, *119*, 273 – 275; *Angew. Chem. Int. Ed.* **2007**, *46*, 269 – 271.

[6] N. E. Polyakov, A. L. Focsan, M. K. Bowman, L. D. Kispert, *J. Phys. Chem. B* **2010**, *114*, 16968 – 16977.

[7] H. A. Frank, C. A. Violette, J. K. Trautman, A. P. Shreve, T. G. Owens, A. C. Albrecht, *Pure & Appl. Chem.* **1991**, *63*, 109 – 114.

[8] T. G. Truscott, *J. Photochem. Photobiol. B: Biol.* **1990**, *6*, 359 – 371.

[9] K. Jørgensen, H. Stapelfeldt, L. H. Skibsted, *Chem. Phys. Lett.* **1992**, *190*, 514 – 519.

[10] H. Kunkely, V. Pawlowski, A. Vogler, *Z. Naturforsch.* **2008**, *63b*, 963 – 967.

[11] V. Pawlowski, H. Kunkely, A. Vogler, *Z. Naturforsch.* **2005**, *60b*, 359 – 362.

[12] M. Buchwald, W. P. Jencks, *Biochemistry* **1968**, *7*, 834 – 843.

[13] V. R. Salares, R. Mendelsohn, P. R. Carey, H. J. Bernstein, *J. Phys. Chem.* **1976**, *80*, 1137 – 1141.

[14] R. P. Ilagan, R. L. Christensen, T. W. Chapp, G. N. Gibson, T. Pascher, T. Polivka, H. A. Frank, *J. Phys. Chem. A* **2005**, *109*, 3120 – 3127.

[15] A. Mortensen, L. H. Skibsted, *Free Rad. Res.* **1997**, *26*, 549 – 563.

[16] R.-M. Han, Y.-X. Tian, Y.-S. Wu, P. Wang, X.-C. Ai, J.-P. Zhang, L. H. Skibsted, *Photochem. Photobiol.* **2006**, *82*, 538 – 546.

[17] S. Sprouse, K. A. King, P. J. Spellane, R. J. Watts, *J. Am. Chem. Soc.* **1984**, *106*, 6647 – 6653.