ELSEVIER

Biophysical Chemistry 56 (1995) 57–62

Biophysical Chemistry

Structure and photobleaching process of chicken iodopsin

Tôru Yoshizawa, Yasushi Imamoto [1]

Department of Information Systems Engineering, Faculty of Engineering, Osaka Sangyo University, 3-1-1, Nakagaito, Daito-shi, Osaka 574, Japan

Abstract

Iodopsin, a dominant cone pigment in a chicken retina, has an absorption spectrum in longer wavelength region than rhodopsin. To account for this red-shift of iodopsin, we had proposed a structural model from retinal analogue experiments, in which iodopsin would have a relatively long distance between the protonated Schiff base nitrogen and the counterion. This was confirmed by a resonance Raman spectroscopy. The photochemical properties of iodopsin were studied and compared with those of rhodopsin, which revealed the following differences. The regeneration rate of iodopsin with 11-*cis*-retinal was 240 times faster than rhodopsin. *Meta*-iodopsin II, the signalling state of iodopsin, decayed about 100 times faster than *meta*-rhodopsin II. The K_m value of *meta*-iodopsin II and rhodopsin kinase was lower than *meta*-rhodopsin II. These results are in consistent with rapid adaptation and low photosensitivity of cones relative to those of rods.

Keywords: Photobleaching; Chicken iodopsin; Rhodopsin

1. Introduction

A chicken retina contains two kinds of visual cells. One is the rod which functions in twilight condition and the other is the cone which functions in daylight. Cones are more rapid but less sensitive in photoresponse than rods [1]. Since light signals are received and transformed by visual pigments, the property of visual pigment may closely correlate to the cell responses. From this standpoint, we have investigated cone visual pigments for the past several years.

Some years ago, we have developed a new method for isolating chicken visual pigments [2], and confirmed that a chicken retina contains one kind of rod pigment, rhodopsin, and four kinds of cone pigments (Fig. 1). The ratio of the visual pigments extracted from the chicken retina with a detergent (a mixture of CHAPS and phosphatidylcholine) was as follows: 40% iodopsin (also called Chicken Red), 5% Chicken Green, 5% Chicken Blue, 1% Chicken Violet and 49% rhodopsin. Since iodopsin not only amounts to about 80% of a total of chicken cone pigments, but also has an absorption spectrum which is in good agreement to a chicken photopic sensitivity curve recorded electrophysiologically [4], iodopsin should be the major photoreceptor protein responsible for the chicken photopic vision. For above reasons, we have studied the structure and photobleaching process of iodopsin intensively.

[1] Present address: Department of Earth and Space Science, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan.

SSDI 0301-4622(95)00015-1

2. Structure of iodopsin

2.1. Primary structure of iodopsin

Using partial peptide analyses on the purified iodopsin together with cDNA analyses, we have determined amino acid sequences of iodopsin [5]. Iodopsin is composed of 362 amino acid residues. The hydropathy index showed that iodopsin has a 7-helical structure like other retinal proteins. The chromophore is binding to Lys-309, and the counterion of the protonated retinylidene Schiff base would be Glu-126. The amino acid identity of iodopsin to Human Red and chicken rhodopsin are 80 and 43%, respectively.

We also determined three other chicken cone pigments [6] and two gecko visual pigments [7]. Then amino acid identities among various animal visual pigments, which had been analyzed so far, were calculated. On the basis of the amino acid identities above 70%, the visual pigments were classified into four groups: L, S, M1 and M2. Surprisingly, each group was composed of visual pigments similar in their absorption spectrum [6]. For instance, group L is in the range of absorption maxima from 521 to 571 nm, including Human Green and Red, Gecko Green (P521), iodopsin (also called Chicken Red) and others. Group M2 composed of Chicken Green, Gecko Blue (P465) and other cone pigments (λ_{max}: 465–511 nm) contains various vertebrate rhodopsins (λ_{max}: 492–503 nm).

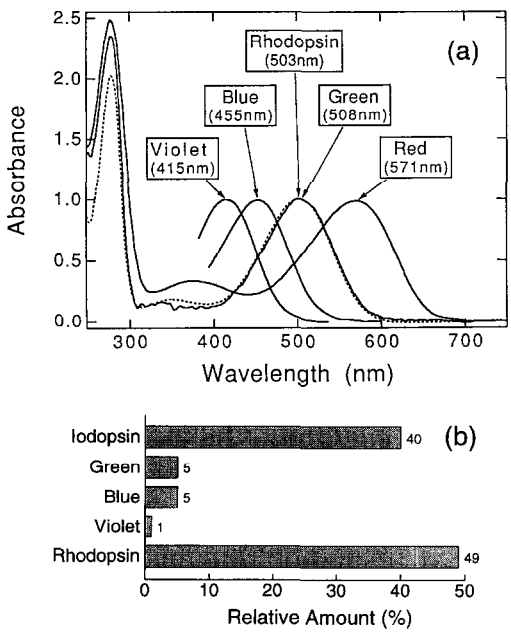

Fig. 1. (a) Absorption spectra of chicken visual pigments [3]. (b) Relative amounts of visual pigments extracted from chicken retinas with the detergent. The amounts were estimated using the absorbances at the absorption maxima.

2.2. Chloride effect of iodopsin

It is well known that iodopsin [8] displays a 'chloride effect' like Gecko Green [9] or halorhodopsin [10]. The absorption maximum of iodopsin is located at 571 nm in the chloride bound form, while it shifts to 530 nm in the chloride depleted form [11]. As far as we have examined, no other visual pigment than group L displays a chloride effect. Thus all the cone pigments belonging to group L would display the chloride effect. In fact, absorption spectra of Human Red and Green expressed in COS cells were recently confirmed to be Cl^--dependent [12]. Since the chloride ions are assumed to bind to positively charged amino acid residues, an alignment of amino acid sequences of rod and cone visual pigments [13] suggests that only 6 amino acid residues (Arg-11, 12, 47, His-194 and Lys-197, 251 in iodopsin) would be candidates for the chloride binding site on the assumption that they are present only in visual pigments belonging to group L, but not to other groups. A recent site-directed mutagenesis experiment on Human Green indicated that the His-197 and Lys-200, corresponding to His-194 and Lys-197 of iodopsin, would be responsible for the chloride effect [12].

2.3. Chromophore–protein interaction in iodopsin

The shape of the chromophore binding site of iodopsin was studied by using retinal isomers (all-*trans*, 13-, 11-, 9- and 7-*cis*-retinals) and various 11-*cis*-fluorinated retinal analogues (14-F-, 12-F-, 10-F- and 8-F-retinals) [14]. As in the case of scotopsin (opsin of rhodopsin), all-*trans*- and 13-*cis*-retinals did not bind to R-photopsin (opsin of iodopsin), while the other isomers and the retinal analogues formed the respective iodopsin isomers and ana-

logues. Thus rhodopsin and iodopsin are almost identical in shape of the retinal binding site to each other. Then opsin shifts in bovine rhodopsin and chicken iodopsin systems were calculated. Irrespective of types of the fluorinated analogues, the difference in opsin shift between both the systems was constant (about 2500 cm^{-1}). Therefore, in the region from the C_8 to the C_{14} position of the chromophore, no electrostatic difference between both the pigments would exist. In the retinal isomers, however, the difference in opsin shift between both the systems was found. On the assumption that the retinylidene chromophore is anchored rigidly at the α-carbon of Lys-309 and loosely at the cyclohexyl ring, we speculated that the counterion of iodopsin is farther from the Schiff base nitrogen than that of rhodopsin. In other words, iodopsin has a Schiff base weakly hydrogen-bonded to the counterion in comparison with rhodopsin.

In order to verify this model, we have examined a vibrational mode of the Schiff base region of iodopsin by low temperature Raman microprobe spectroscopy [15]. The C=NH stretching mode of the mixture of iodopsin and isoiodopsin appeared at 1644 cm^{-1}, which shifted down to 1621 cm^{-1} in D_2O buffer. It has been suggested that the wave number of retinylidene Schiff base is sensitive to the C=N bond order and the C=NH rock-stretching coupling. When the nitrogen is deuterated, the rock-stretch coupling can be removed. Thus it was shown that the C=ND stretching wave numbers linearly decrease with increase of absorption maxima, suggesting that at least part of opsin shift in visual pigments results from weakened electrostatic interaction between the retinal chromophore and its protein counterion. Thus these results are consistent with the model presented previously.

2.4. Regeneration rate of cone pigments

It has been reported that the rate of regeneration of iodopsin (the binding rate of R-photopsin with 11-*cis*-retinal) is 500 times faster than bovine rhodopsin [16]. Recently, we confirmed this phenomenon using the purified R-photopsin and chicken scotopsin samples [17]. Moreover, to verify that the rapid regeneration is a common property among cone pigments, the binding rate of G-photopsin (the protein moiety of Chicken Green) was estimated [17] (Fig. 2).

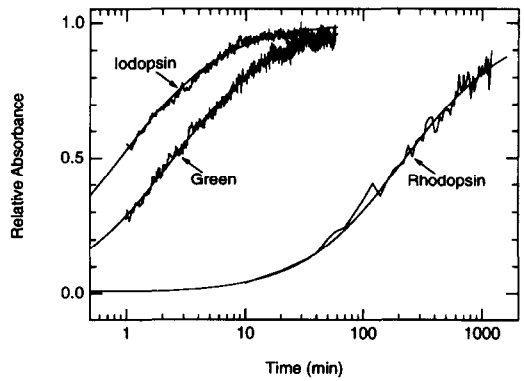

Fig. 2. Regeneration of iodopsin, Chicken Green, and chicken rhodopsin. An equimolar amount of 11-*cis* retinal (7.5 μM, 5 μl) was added to R-photopsin, G-photopsin, or chicken scotopsin (150 nM, 250 μl) at 2° C. Then each sample was incubated at 2° C and its absorbance increase was monitored at 570 nm (R-photopsin) or 530 nm (G-photopsin and scotopsin). The curves were fitted with hyperbolic curves whose time constants were 1 min, 2.5 min, and 4 h (R-photopsin, G-photopsin and scotopsin, respectively) (modified from Shichida et al. [17]).

The regeneration rate constants of iodopsin and Chicken Green were 240 and 100 times faster than those of chicken rhodopsin, indicating that the cone pigments bind 11-*cis*-retinal much faster than rhodopsin. The cone pigments function under daylight conditions and the rapid regeneration after absorption of a photon could be essential for cone pigments.

3. Photobleaching process of iodopsin

For many years, only three species of intermediates of iodopsin (batho-, lumi- and *meta*-iodopsins) were described, which were detected by irradiation at low temperatures [18,19]. Several years ago, we found that these lumi- and *meta*-iodopsins contained a considerable amount of 7-*cis* products [20]. In addition, bathoiodopsin formed by irradiation of iodopsin at liquid nitrogen temperature recovered to the original iodopsin on warming above $-170°$ C [11,18]. For elucidating the photobleaching process of iodopsin, it is difficult to do low-temperature experiments. Instead, laser flash photolysis techniques were applied.

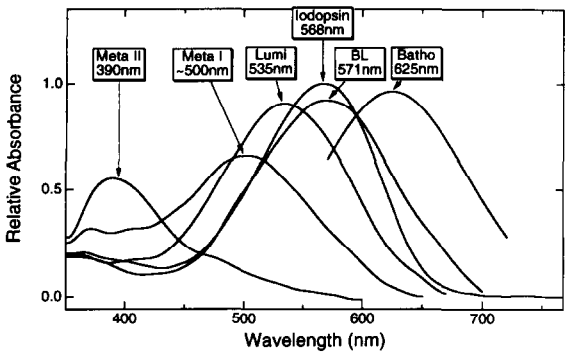

Fig. 3. Absorption spectra of iodopsin and its photo-bleaching intermediates at 20° C. The absorption maxima of batho-, BL-, lumi-, *meta*-I-, and *meta*-II-iodopsins were estimated to be 625, 571, 535, 500, and 390 nm, respectively (modified from Shichida et al. [23]).

3.1. Primary photochemical reaction of iodopsin

In order to confirm whether or not bathoiodopsin is formed at physiological temperature, we carried out a picosecond laser photolysis experiment of iodopsin [21]. Iodopsin was excited by using a 21 ps laser pulse at 532 nm, and the transient spectra were recorded from 15 ps to 1 ns after the excitation. The red-shifted spectra (difference maximum: 650 nm) were obtained, which were not changed in ps time scale. It indicated that bathoiodopsin was formed at room temperature, and was stable in the ps time scale. The spectra of bathoiodopsin (λ_{max}, 625 nm) at room temperature are shown in Fig. 3.

Recently, we have excited iodopsin with a 0.5 ps laser pulse at 600 nm [22], and succeeded to record a spectrum of the excited state of iodopsin having an absorption maximum at shorter wavelength than that of iodopsin. Excited iodopsin decayed to photoiodopsin with a lifetime of 0.2 ps. Therefore, the first intermediate of iodopsin should be photoiodopsin. Photoiodopsin decayed to bathoiodopsin with a lifetime shorter than 10 ps.

3.2. Nanosecond laser photolysis of iodopsin

For detection of lumi- and *meta*-iodopsins, iodopsin was excited with a 17 ns laser pulse at 560 nm [23]. Then the transient difference spectra were recorded in ns to ms time scales.

Just after the excitation, a red-shifted product was formed, which was assigned to be BL iodopsin, because its difference absorption spectra were located at a shorter wavelength than that of bathoiodopsin. The decay process of BL-iodopsin was composed of three steps of blue-shift. The spectral change of the first step formed an isosbestic point at 550 nm, whose kinetics were expressed by a single exponential curve with a time constant of 130 ns. The second spectral change was recorded in a μs time scale, in which a rough isosbestic point was observed at about 500 nm. The kinetic profiles were expressed by two sequential single-exponential curves having time constants of 230 μs and 6 ms. These results indicate that lumiiodopsin changes to *meta*-iodopsin I with a time constant of 230 μs and then to a product having an absorption maximum at 390 nm (390-product) with a time constant of 6 ms.

Then the absolute absorption spectra of iodopsin intermediates were calculated from the transient difference spectra by estimating the ratio of the constituent intermediates on the basis of their lifetimes. The calculated absorption spectra of batho-, BL-, lumi-, *meta*-I iodopsins and 390-product (drawn in Fig. 3 as *meta*-II-iodopsin) are shown in Fig. 3. Their absorption maxima were estimated to be 625, 571, 535, 500, and 390 nm, respectively.

3.3. Detection of meta-iodopsin II

There is a possibility that the 390-product might not be *meta*-iodopsin II but all-*trans*-retinal. In order to exclude this possibility, we have recorded circular dichroisms (CD) of iodopsin intermediates [24]. Bovine *meta*-rhodopsin II has an optical activity, while all-*trans*-retinal and *meta*-rhodopsin III have no CD signal [25], so that if 390-product of iodopsin may have a CD signal, one can assign it to be *meta*-iodopsin II.

An iodopsin sample was irradiated with an orange light at 570 nm for 30 s at $-10°$ C. Then an absorption spectrum was recorded which showed an absorption maximum at about 390 nm and a shoulder at about 470 nm. This indicates that the spectrum should be a mixture of the 390-product and *meta*-iodopsin I. A CD spectrum of the same sample displayed a broad positive band at 400 nm. Finally the sample was kept in the dark for 60 min at

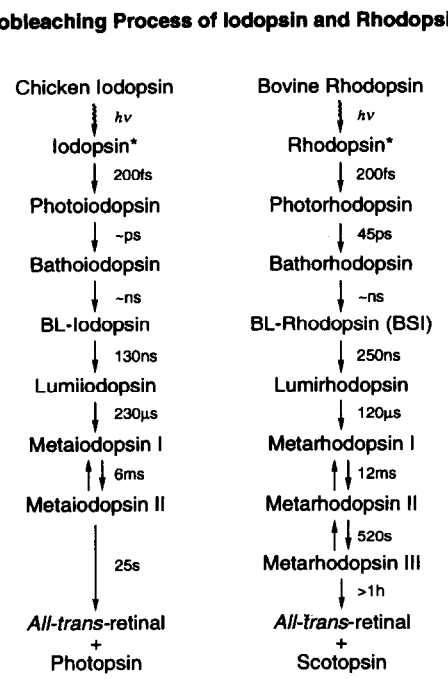

Fig. 4. Comparison of photobleaching process of chicken iodopsin to that of bovine rhodopsin. The lifetime of intermediates are indicated at the side the arrows (modified from Shichida et al. [23] and Yoshizawa [13]).

$-10°$ C. The absorbance at 390 nm increased with disappearance of the shoulder at 470 nm, and only a small CD from 350 nm to 600 nm was recorded. Thus this sample should be regarded as all-*trans*-retinal. Accordingly, 390-product having the CD signal should be *meta*-iodopsin II.

Using the CD signal at 380 nm, we have measured lifetimes of *meta*- II intermediates of bovine and chicken rhodopsins and iodopsin. Rhodopsins and iodopsin were irradiated for 30 s at wavelengths longer than 500 nm and 570 nm, respectively, at temperatures from -10 to 15° C. From the Arrhenius plot, the time constants of *meta*-iodopsin II and chicken and bovine *meta*-rhodopsin II at 20° C were estimated to be 25, 210 and 520 s, respectively.

The photobleaching processes of chicken iodopsin in comparison with that of bovine rhodopsin are presented in Fig. 4. The bleaching of iodopsin intermediates are faster than those of bovine rhodopsin. Among many intermediates, the most noticeable intermediate in connection with the cascade systems would be *meta*-II intermediates.

In order to get conclusive evidence that *meta*-iodopsin II interacts with transducin [26], we have examined the effect of bovine transducin in the presence and absence of GTP on the thermal decay of *meta*-iodopsin II [24]. The decay of *meta*-iodopsin II was remarkably suppressed by adding only transducin to the sample like in the case of *meta*-rhodopsin II. When the sample contained both transducin and GTP, the decay of *meta*-iodopsin II was almost identical to the sample without transducin. These experiments clearly show that *meta*-iodopsin II interacts with transducin.

3.4. Quenching mechanism of meta-iodopsin II

In rhodopsin the activation of the enzymatic cascade system by *meta*-rhodopsin II is quenched by phosphorylation of the C-terminal domain of its apoprotein. Because iodopsin also has possible phosphorylation sites in the C-terminus region, its quenching process would be similar to that of rhodopsin. We studied the phosphorylation of iodopsin using bovine rhodopsin kinase [27]. The K_m value of iodopsin was about 3 times lower than that of rhodopsin. This result suggests that the affinity of iodopsin to kinase is higher than rhodopsin and the quenching of *meta*-iodopsin II would take place faster than *meta*-rhodopsin II. This observation and the fast decay of *meta*-iodopsin II relative to *meta*-rhodopsin II are in agreement with the fact that the cone photoresponses are shut-off more rapidly than those of rods.

3.5. Thermal recovery of iodopsin from meta-iodopsin I

During the investigation of the thermal behavior of *meta*-iodopsins at $-20°$ C, we encountered unexpected thermal behavior of *meta*-iodopsin I. Namely, in the thermal decay process of *meta*-iodopsin I, the generation of original iodopsin was observed [28]. We studied the generation process of iodopsin in detail and revealed that about one half of *meta*-iodopsin I thermally reverts to iodopsin (Fig. 5). Because no reverse reaction takes place in the photobleaching process of the other visual pigments, it

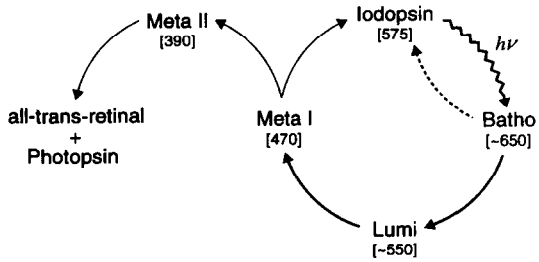

Fig. 5. Scheme for the photobleaching process of iodopsin at −20° C. Wavy line and straight lines show the photoreaction and the thermal reactions, respectively. About one half of metaiodopsin I reverts to iodopsin thermally. The absorption maxima are indicated in the brackets (modified from Imamoto et al. [28]).

would be a unique property of iodopsin. We have not yet examined whether this thermal recovery takes place in the physiological condition and cannot state its physiological importance. However, if about half of *meta*-iodopsin I reverts to iodopsin in vivo, the net quantum yield of iodopsin would be reduced by 50%. Moreover, if the extent of the thermal recovery is changed by the condition in the cell, it could be involved in regulation of the photosensitivity. This reverse reaction of *meta*-iodopsin will be one of the most attractive features of iodopsin, subject to our future research.

Acknowledgements

This work was supported in part by a grant from the Human Frontier Science Program, by a Grant-in-Aid for JSPS fellows and by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan.

References

[1] D.A. Baylor and B. Nunn, Methods Enzymol., 81 (1982) 403.
[2] T. Okano, Y. Fukada, I.D. Artamonov and T. Yoshizawa, Biochemistry, 28 (1989) 8848.
[3] T. Yoshizawa and Y. Fukada, in P.A. Hargrave (Editor), Methods in Neurosciences 15, Academic press, 1993, p. 161.
[4] T. Yoshizawa, Biophys. Chem., 50 (1994) 17.
[5] O. Kuwata, Y. Imamoto, T. Okano, K. Kokame, D. Kojima, H. Matsumoto, A. Morodome, Y. Fukada, Y. Shichida, Y. Yasuda, Y. Shimura and T. Yoshizawa, FEBS Lett., 272 (1990) 128.
[6] T. Okano, D. Kojima, Y. Fukada, Y. Shichida and T. Yoshizawa, Proc. Natl. Acad. Sci. USA, 89 (1992) 5932.
[7] D. Kojima, T. Okano, Y. Fukada, Y. Shichida, T. Yoshizawa and T.G. Ebrey, Proc. Natl. Acad. Sci. USA, 89 (1992) 6841.
[8] A. Knowles, Biochem. Biophys. Res. Commun., 73 (1976) 56.
[9] F. Crescitelli, in F. Crescitelli (Editor), Handbook of Sensory Physiology VII/5, Springer-Verlag, 1977, p. 391.
[10] T. Ogurusu, A. Maeda, N. Sasaki and T. Yoshizawa, Biochim. Biophys. Acta, 682 (1982) 446.
[11] Y. Imamoto, H. Kandori, T. Okano, Y. Fukada, Y. Shichida and T. Yoshizawa, Biochemistry, 28 (1989) 9412.
[12] Z. Wang, A.B. Asenjo and D.D. Oprian, Biochemistry, 32 (1993) 2125.
[13] T. Yoshizawa, Photochem. Photobiol., 56 (1992) 859.
[14] Y. Fukada, T. Okano, Y. Shichida, T. Yoshizawa, A. Trehan, D. Mead, M. Denny, A.E. Asato and R.S.H. Liu, Biochemistry, 29 (1990) 3133.
[15] S.W. Lin, Y. Imamoto, Y. Fukada, Y. Shichida, T. Yoshizawa and R.A. Mathies, Biochemistry, 33 (1994) 2151.
[16] G. Wald, P.K. Brown and P.H. Smith, J. Gen. Phys., 38 (1955) 623.
[17] Y. Shichida, H. Imai, Y. Imamoto, Y. Fukada and T. Yoshizawa, Biochemistry, 33 (1994) 9040.
[18] T. Yoshizawa and G. Wald, Nature, 214 (1967) 566.
[19] R. Hubbard and A. Kropf, Nature, 183 (1959) 448.
[20] Y. Imamoto, Y. Shichida, Y. Fukada and T. Yoshizawa, Comp. Phys. Biochem., 8 (1991) 160.
[21] H. Kandori, T. Mizukami, T. Okada, Y. Imamoto, Y. Fukada, Y. Shichida and T. Yoshizawa, Proc. Natl. Acad. Sci. USA, 87 (1990) 8908.
[22] Y. Shichida, H. Kandori, Y. Imamoto, H. Imai, T. Yoshizawa and K. Yoshihara, Biophysics (suppl.), 33 (1993) 82, in Japanese.
[23] Y. Shichida, T. Okada, H. Kandori, Y. Fukada and T. Yoshizawa, Biochemistry, 32 (1993) 10832.
[24] T. Okada, T. Matsuda, H. Kandori, Y. Fukada, T. Yoshizawa and Y. Shichida, Biochemistry, 33 (1994) 4940.
[25] T. Yoshizawa and S. Horiuchi, in H. Langer (Editor), Biochemistry and Physiology of Visual Pigments, Springer-Verlag, Heidelberg, 1973, p. 69.
[26] Y. Fukada and T. Yoshizawa, FEBS Lett., 146 (1982) 117.
[27] Y. Fukada, K. Kokame, T. Okano, Y. Shichida, T. Yoshizawa, J.H. McDowell, P.A. Hargrave and K. Palczewski, Biochemistry, 29 (1990) 10102.
[28] Y. Imamoto, H. Imai, T. Yoshizawa and Y. Shichida, FEBS Lett., 354 (1994) 165.