THERMAL DENATURATION OF OPSIN IN WARM-BLOODED ANIMALS AS A POSSIBLE MECHANISM OF LIGHT-INDUCED RETINAL DAMAGE

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Prolonged exposure to a high intensity of illumination leads to impairment or even loss of vision [10]. The molecular mechanism of this impairment of vision in light-induced retinal injury is not yet clear. An important role in the pathogenesis of this disease may be played by lipid peroxidation (LPO): Administration of antioxidants *in vivo* prevented impairment of vision [4]. There is reason to suppose that a definite place in the genesis of light-induced retinal injury is occupied by thermal denaturation of the photoreceptor protein (PRP) opsin. Under ordinary, nonextremal conditions of illumination the greater part of the PRP in the retina is in the nondecolorized state, associated with 11-cis-retinal. In this form the PRP rhodopsin is extremely resistant to thermal denaturation [1]. Under extremal conditions of illumination, when a high proportion (up to 50-80%) of the PRP in the eye is in the form of opsin (PRP in the decolorized state, lacking the chromophore) and when, in addition, LPO may be induced in the photoreceptor [3], the denaturation temperature of the PRP of vertebrates falls to 15-16°C [1, 2]. Under these conditions the rate of thermal denaturation of the PRP must be increased by 4 to 6 orders of magnitude [1], and this may lead to the accumulation of denatured opsin in the photoreceptor membranes (PRM). Data in the literature on the kinetics of thermal denaturation of rhodopsin and opsin [5, 6, 8, 9, 13] cannot be used in the calculation of the velocity of thermal denaturation of opsin in native PRM at the physiological temperature (37°C), because there is reason to suppose that all these data were obtained in the study of preparations of PRM enriched with LPO products [1].

In the investigation described below the velocity of thermal denaturation of PRP (opsin and rhodopsin) of warm-blooded animals was studied in native PRM and under conditions of LPO induced in the PRM at different temperatures in order to be able to evaluate the rate of thermal denaturation of opsin in the retina at the physiological temperature.

EXPERIMENTAL METHOD

The test object consisted of fractions of bovine [6] and Wistar rat PRM [12]. To prevent LPO and oxidation of SH-groups in the PRM preparations all the solutions used for preparative isolation of PRM and to measure the velocity of thermal denaturation of PRM in native PRM contained 0.15 mM EDTA, 1 mg/liter of ionol, and 1 mM dithiothreitol. The kinetics of thermal denaturation of rhodopsin in PRM was measured at pH 7.1, just as in [1, 8]. LPO in the fractions of PRM was induced in a Fe^{++}–ascorbate system as described previously [9, 13]. Decolorization of the PRM fraction to obtain a preparation of opsin was carried out at pH 6.0 by green light (500 nm) for 20 min at 0°C. Denaturation of opsin was judged from disappearance of its ability to bind with 11-cis-retinal with the formation of rhodopsin. Rhodopsin in the fraction of declorized PRM was regenerated for 20 min at 37°C in 0.05 M phosphate buffer, pH 7.1, with 11-cis-retinal and opsin in the ratio of 10:1. The PRM were incubated to measure the kinetics of denaturation of opsin and rhodopsin in a water ultrathermostat in complete darkness to avoid photodenaturation of PRP. All the work was done on freshly isolated preparations of PRM.

EXPERIMENTAL RESULTS

The kinetics of thermal denaturation of PRP (data not given), as well as the Arrhenius plots for the velocity of thermal denaturation of opsin and rhodopsin in bovine and rat PRM

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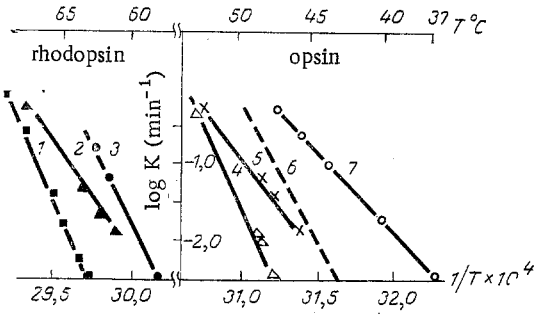

Fig. 1. Effect of temperature on velocity of thermal denaturation of bovine and rat opsin and rhodopsin in native PRM and in PRM subjected to LPO. Top abscissa — temperature of incubation medium (in °C); bottom abscissa — reciprocals of absolute temperature multiplied by 10^4; ordinate, $\log_{10}$ of velocity constant of denaturation of bovine and rat opsin and rhodopsin (in min^{-1}). Incubation of PRM in 0.05 M phosphate buffer, pH 7.1. 1) Arrhenius plot for bovine rhodopsin in native PRM, 2) Arrhenius plot for bovine rhodopsin, in good enriched with LPO products, 3) rat rhodopsin in native PRM, 4) bovine opsin in native PRM, 5) bovine opsin in PRM enriched with LPO products, 6) rat opsin in native PRM (calculated data), 7) rat opsin in PRM subjected to LPO.

were linear over the whole range of temperatures studied: from 37 to 70°C (Fig. 1). The activation energy of thermal denaturation of E_1 rhodopsin and opsin in native bovine PRM were equal to one another, namely about 190 kcal/mole (Table 1). The denaturation temperature of bovine opsin in native PRM was found to be 15.7°C lower than that of bovine rhodopsin, in good agreement with the results of direct microcalorimetric measurements [1, 2]. Induction of LPO in preparations of bovine PRM evoked a marked decrease in the resistance of opsin to heat, as shown by a decrease in E_a of bovine opsin from 185 to 118 kcal/mole and, in addition, by a fall of the denaturation temperature of opsin from 51 to 49°C. Under the influence of induced LPO the value of E_a of bovine rhodopsin was lowered to the level determined in previous investigations [5, 8, 9, 13], namely above 100 kcal/mole. Since all tests of the properties of PRP in native PRM in the present investigation were done on fresh preparations of PRM, and since measures were adopted to prevent the formation of LPO products in the process of preparative isolation of the PRM fractions, the value of E_a for rhodopsin and opsin obtained, namely 190 kcal/mole, evidently characterizes the native state of PRP in bovine PRM, whereas the value of 110-100 kcal/mole obtained for bovine rhodopsin in a number of previous investigations [5, 6, 8, 9, 13], evidently characterizes bovine PRM enriched with LPO products. Agreement between the values of E_a for rhodopsin in fresh preparations of PRM and in PRM preparations kept at the temperature of liquid nitrogen [1] indicates that if PRM are stored in liquid nitrogen the thermal stability of PRM remains the same as in fresh preparations of native PRM.

The denaturation temperature of rat rhodopsin in native bovine PRM was 4.7°C below that of bovine rhodopsin, but E_a for rat rhodopsin had the same value as for bovine rhodopsin. Correspondingly, the velocity of thermal denaturation of rat rhodopsin in native PRM was more than 100 times greater than that of bovine rhodopsin (Table 1). Illumination led to a sharp decrease in E_a of rat PRP, whereas under these circumstances E_a of rat opsin was equal in value to E_a of bovine opsin after the action of LPO on bovine PRM (120 kcal/mole). It can be tentatively suggested that under these experimental conditions illumination of rat PRM, unlike bovine PRM, was accompanied by LPO and that the kinetic parameters obtained characterize, not native rat PRM, but PRM exposed to the action of LPO.

The rate of denaturation of opsin in decolorized rat PRM was measured actually at 37°C, and in all other cases (native bovine and rat PRM, bovine PRM after induction of LPO in them) the velocity of denaturation of PRP at the physiological temperature of 37°C was calculated by extrapolation of an Arrhenius plot to 37°C. The velocity of thermal denaturation of opsin in native rat PRM at 37°C was calculated by using an Arrhenius plot for thermal denaturation of rat rhodopsin in native PRM for extrapolation (Fig. 1) and assuming that, just as in bovine PRM, decolorization of rat PRP in native PRM lowers its denaturation temperature by 15°C but does not change its E_a. The time during which 5 and 50% of the total opsin present in PRM undergoes denaturation in bovine and rat PRM also was calculated. The value of 5% was chosen as conventional criterion of "worsening of the quality" of PRM, for we know that de-

TABLE 1. Kinetic Parameters of Thermal Denaturation of Rhodopsin and Opsin in PRM of Bovine and Rat Retina

Kinetic parameters of thermal denaturation of rhodopsin and opsin	Native bovine PRM		BovinePRM+LPO	Native rat PRM		Illumination of rat PRM+ LPO, opsin
	rhodopsin	opsin	opsin	rhodopsin	opsin (calc.)	
E_a of thermal denaturation of PRP, kcal/mole	195±10	185±15	118±8	176±6	176	120±15
Denaturation temp. of PRP* (in °C)	66,7	51	49,3	62	47	43,7
Log_{10} of velocity constant of denaturation of PRP at 37°C, min^{-1}	—13	—6,8	—4,3	—10,4	—5,0	—3,0±0,5
Time during which 5% of PRP decomposes at 37°C	10^6 years	220 days	18 h	2500 years	$3^1/_2$ days	1 h
Time during which 50% of PRP decomposes at 37°C	—	—	10 days	—	48 days	11 h

Legend. *Temperature at which 50% of PRP denatures in 10 min.

colorization of 5% of rhodopsin lowers the threshold sensitivity of the retina by 10 times [7]. As may be seen from the data given in Table 1, rat opsin in native PRM decomposes at a rate commensurate with the rate of its resynthesis during renewal of the disks of the outer segment of the rod: 5% of rat opsin denatures in 3.5 days, whereas renewal of the total PRP takes 9 days [14]. The highest rate of denaturation of opsin was observed in decolorized rat PRM and also in decolorized bovine PRM subjected to LPO (Table 1). The results are evidence that under extremal conditions (a high level and long duration of illumination, induction of LPO) the velocity of thermal denaturation of PRP may exceed the velocity of renewal of PRP in the photoreceptor or may be commensurate with it [14]. Consequently, the increased level of illumination and the relatively high content of opsin in the eye of animals, like the increased content of LPO products, the depressed antioxidant activity, and the delayed regeneration of rhodopsin, may lead to accumulation of denatured opsin in PRM of the retinal photoreceptor. We postulate that accumulation of denatured opsin in PRM may be one of the direct causes of the impairment of vision under conditions of increased intensity of illumination. This hypothesis is in good agreement with the fact that raising an animal's body temperature causes light-induced retinal injury to develop much faster [11].

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