

MOLECULAR CHANGES OF THE MEMBRANE EMBEDDED CARBOXYL GROUP GLU122 OF BOVINE RHODOPSIN DURING THE TRANSITION TO THE ACTIVE STATE METARHODOPSIN-II: AN INVESTIGATION ON THE GLU122->ASP MUTANT USING FT-IR DIFFERENCE SPECTROSCOPY

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The visual pigment rhodopsin belongs to the class of G-protein coupled receptors. Its light sensitive part, the chromophore 11-cis-retinal, is bound to a lysine of the protein via a protonated Schiff base. Photoisomerization of the chromophore to the all-trans geometry transforms rhodopsin into an active state, called metarhodopsin-II (MII), in which the Schiff base is deprotonated. This state binds the visual G-protein transducin and thereby triggers the signal transduction cascade¹. It was shown that this deprotonation step is essential for rhodopsin activation². Biochemical studies (e.g. ref.³) on recombinant rhodopsins have shown that Glu113 acts as a counterion for the positive charge of the protonated Schiff base. Membrane embedded carboxyls may well serve as groups participating in proton exchange with the Schiff base. This was especially demonstrated for another retinal protein, the light-driven proton pump bacteriorhodopsin⁴. Therefore, it is of special interest to investigate the molecular changes of internal carboxyl groups by FT-IR difference spectroscopy. This method, which allows the detection of molecular changes of the chromophore and the protein, was applied to the photoreaction of rhodopsin⁵. The difference spectra obtained for the formation of MII showed strong bands which could be assigned to internal carboxyl groups. But it was not possible to assign these bands to specific groups or to interpret them at a molecular level. In this contribution we show batho and MII difference spectra of a rhodopsin mutant in which one internal carboxyl group, Glu122, is replaced by an Asp. Thus, the functional group of this amino acid is retained, but it can be expected, that, if in the difference spectrum bands due to Glu122 are present, these bands will experience some alterations by this replacement. Comparing the spectra of wild type and mutant rhodopsin will allow the assignment of bands due to Glu122 and the characterization of its molecular changes. Since the mutants were expressed in COS-cells in low quantity, they had to be purified in dodecylmaltoside³. Correspondingly, the FT-IR

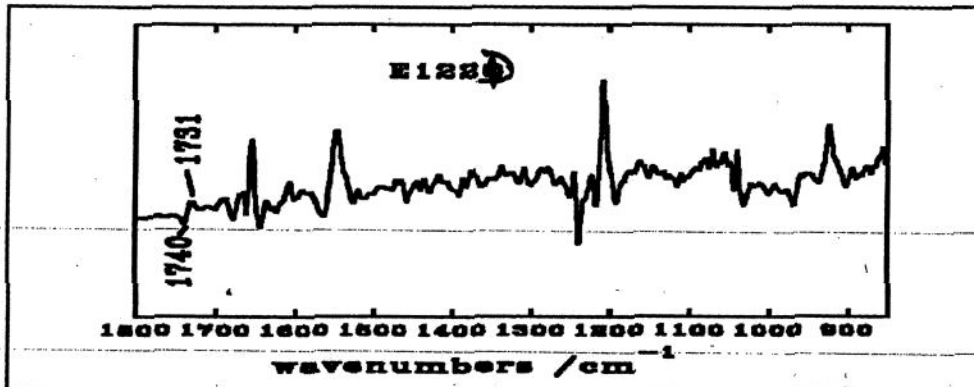


Fig. 1 Rhodopsin->bathorhodopsin difference spectrum of the Glu122->Asp mutant.

measurements were also performed in this detergent. In order to obtain the difference spectra of the MII state, single beam spectra before and after illumination were collected at 0 °C. The pH of the infrared sample was adjusted to 5.5. For the batho spectra, the temperature was 80 K. Control measurements showed that almost identical difference spectra were obtained for rhodopsin in detergent and rhodopsin in membranes. Biochemical studies on this mutant demonstrated increased activity for transducin activation and an absorption maximum blue-shifted by 25 nm³. Fig. 1 shows the batho spectrum of the mutant. With the exception of the

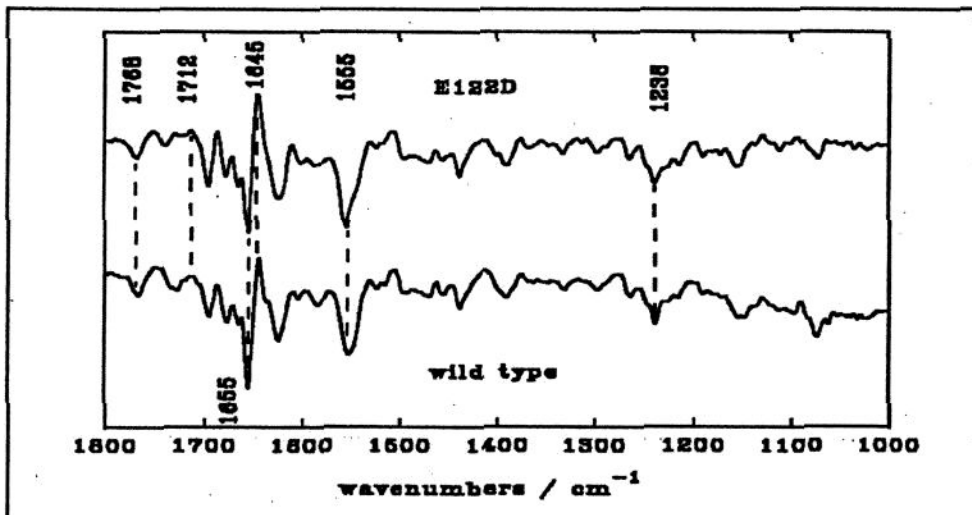


Fig. 2 Rhodopsin->Metarhodopsin II difference spectra of wild type rhodopsin and of the Glu122->Asp mutant

difference band $1740/1731\text{ cm}^{-1}$, the spectrum is in excellent agreement with spectra of wild type rhodopsin⁵. However, no strong bands are seen in the latter above 1700 cm^{-1} . Thus, the new difference band is due to Asp122, replacing the former glutamic acid. The band shows that upon the transition to bathorhodopsin, this new aspartic acid is considerably influenced. Therefore, it appears that this group interacts more strongly with the chromophore, which may explain the blue-shifted absorption maximum.

Fig. 2 compares the spectra of wild type rhodopsin (expressed in COS-cells) and of the Glu122→Asp mutant. The spectral range between 1700 and 1000 cm^{-1} exhibits

practically no deviations. This demonstrates that the retinal binding site is not distorted by the mutation, and that the conformational changes necessary for transducin activation are not abolished. This is in agreement with the activation studies. However, as in the case of the batho spectrum, characteristic deviations can be seen above 1700 cm^{-1} (Fig. 3). The positive band at 1745 and the two negative bands at 1734 and 1727 cm^{-1} disappear and, instead, a difference band at $1740/1729\text{ cm}^{-1}$ can be

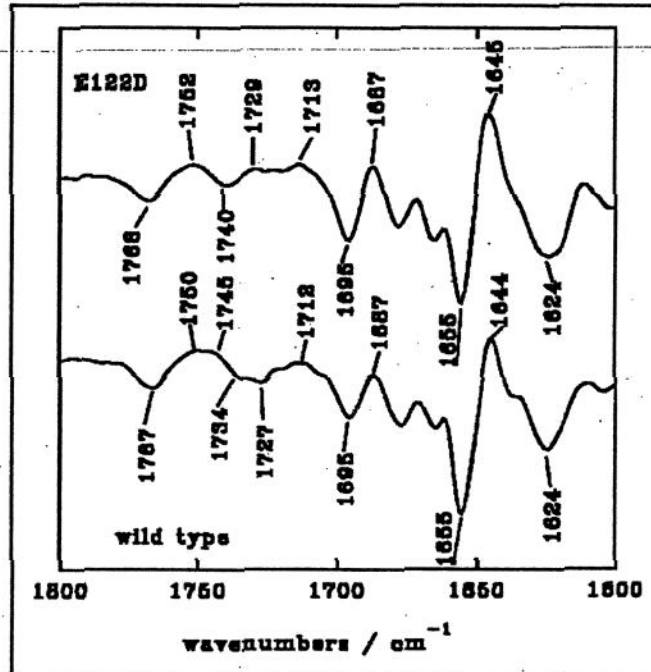


Fig. 3 Enlarged part of the spectral range above 1600 cm^{-1} of Fig. 2

seen. This difference band is similar to that observed in the batho spectrum. Thus, this new band can be assigned to Asp122. Similar as in batho, this group is stronger hydrogen bonded in MII. From the disappearance of the band structure $1734/1745\text{ cm}^{-1}$ in the wild type spectrum, it can be deduced that it is caused by Glu122. In contrast to the aspartic acid, the glutamic acid is less hydrogen bonded in MII. It is surprising that also the second negative band, at 1727 cm^{-1} , disappears in the mutant. This band was previously assigned to an amide-I band caused by a strongly twisted C-N peptide bond. However, the results on the mutant would, at first sight,

suggest that this band is also caused by Glu122. However, it is difficult to imagine that two C=O bands are due to a single carboxyl group. It is interesting to note that Glu122 is preceded by two glycines, which are known to destabilize helical structures. Thus, it might be that the mutation at position 122 influences also the backbone structure in this region.

Since Glu113 is the counterion, i.e. it is deprotonated, the only interior carboxyl group left is Asp83. Thus, the results suggest that the difference band at $1767/1750\text{ cm}^{-1}$ is caused by Asp83. If this is correct, the practically unchanged bandstructure observed in the mutant spectrum indicates that Glu122 and Asp83 do not interact with each other. Since both, the bands of the photoproduct and of the initial state absorb above 1700 cm^{-1} , it can be concluded that Glu122 and, probably, Asp83, are protonated in rhodopsin and that they do not change their protonation state in MII, i.e. they do not take part in a proton relay system involved in Schiff base deprotonation. Similar conclusions could be deduced from our measurements on the Asp83→Asn and Glu112→Gln mutants (PNAS, in press).

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