

Receptor-Bound Conformation of α -Peptide of Transducin (G_t) is not Stabilized by a “ π -Cation” Interaction but by Constrained Lactam Bridges Between Residues 341 and 350

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Introduction

Light-induced activation of rhodopsin (R^*) leads to its conformation change and the binding of transducin G_t . Synthetic $G_t\alpha$ (340-350) peptide has been demonstrated to stabilize R^* as does G_t . The bound conformation of R^* -bound $G_t\alpha$ (340-350) has been determined by TrNOE NMR measurements [1]. The adjacent disposition of the ϵ -amino group of Lys-341 toward the side-chain phenyl ring of Phe-350 suggests a possible π -cation interaction. To investigate this π -cation hypothesis, we measured the affinity with R^* of a series of α -peptide analogs with different *para*-substituents on the Phe-350 phenyl ring. In order to further exploit the proximity between the side chains of Lys-341 and Phe-350, we also prepared α -peptide analogs with straightforward lactam bridges between the side chains at 341 and 350.

Results and Discussion

H-Ile-Lys-Glu-Asn-Leu-Lys-Asp-Cys-Gly-Leu-Xxx-OH

Xxx = Tyr (1); Trp (2); Phe(*p*-F) (3); Phe(*p*-NH₂) (4); Phe(*p*-NO₂) (5); Cha (6)

H-Ile-cyclo(Xxx-Glu-Asn-Leu-Lys-Asp-Cys-Gly-Leu-Yyy)-OH

Xxx = Lys (7), Lys (8); Yyy = Asp (7), Glu (8)

Xxx = Glu (9), Asp (10); Yyy = Phe(*p*-NH-) (9), Phe(*p*-NH-) (10)

A series of α -peptide analogs with varied *para*-substituents on the side-chain aromatic ring of Phe-350 were synthesized: with electron-donating groups, *i.e.* 350-Tyr (1), 350-Trp (2), 350-Phe(*p*-NH₂) (4); with electron-withdrawing groups, *i.e.* 350-Phe(*p*-F) (3), 350-Phe(*p*-NO₂) (5) and a side chain with no π system, *i.e.* 350-Cha (6). The postulated π -cation interaction consists of an interaction between a side chain NH₃⁺ cation from 341-Lys and the varied side-chain aromatic systems on 350. If this interaction exists, a stronger π -cation interaction would constrain the peptide into a more favorable binding conformation and stabilize the cation in the compact hydrophobic pocket resulting in higher binding affinity, *vice versa*. However, binding affinity of these analogs did not show this expected correlation with electronic effect, but correlate closely to the hydrophobicity of the side chain modification of 350. Highly hydrophilic side chains like 350-Phe(*p*-NH₂) (4) and 350-Tyr (1) have only retained 33 and 55% of affinity. 350-Phe(*p*-F) (3), 350-Phe(*p*-NO₂) (5) and 350-Trp (2) show comparable affinity due to similar hydrophobic side chains. 350-Cha (6) possesses the most hydrophobic side chain and exhibits 1.5-fold higher affinity than $G_t\alpha$ (340-350). Therefore, the π -cation interaction does not seem to be involved in the case of the α -peptide interacting with R^* , while the hydrophobicity of the side chains appears a more significant driving force. The fact that increased hydrophobicity on the side chain of 350 displays stronger binding affinity confirms the hydrophobic role of side chain at position 350, determined by TrNOE measurements, as an important component of a hydrophobic patch when interacts with the hydrophobic core of R^* . A com-

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binatorial approach using phage display and random residue mutation has found that substitution of the cationic 341-Lys with a neutral Leu increases affinity [2]. This implies that the side chain of 341-Lys probably undergo deprotonation upon binding to R*. The energetic cost of deprotonation and desolvation of the ϵ -amine may allow control of the affinity to maintain the appropriate dissociation rates for the activated complex in its biological context. This hypothesis is in good agreement with the lack of evidence for a π -cation interaction from this study despite of the proximity of the ϵ -amine “cation” and the Phe aromatic system.

We also tried direct lactam-bridge construction between the side chains at these two positions. Four cyclic analogs were prepared: cyclo(KD) (**7**), cyclo(KE) (**8**), and cyclo(EF(NH₂)) (**9**), cyclo(DF(NH₂)) (**10**). Both cyclo(KD) (**7**) and cyclo(KE) (**8**) display enhanced binding affinity, 1.67- and 4.17-fold respectively. However, in case of cyclization with the *para*-aminophenyl ring of residue 350, cyclo(DF(NH₂)) (**10**) totally loses its binding affinity, while cyclo(EF(NH₂)) (**9**) exhibits 10-fold enhanced binding affinity. The fact that cyclic peptides with a lactam linkage between side chains at 341 and 350 show higher affinity even with the loss of a hydrophobic phenyl ring suggests that this configuration is favored in binding to R* and supports the TrNOE G₁ α structure. The further increased affinity after the addition a phenyl ring back into the cyclic peptide again agrees well with the significant hydrophobic contribution on residue 350 for binding. The total loss of affinity of cyclo(DF(NH₂)) (**10**) suggests this bound conformation appears rather specific as removal of one methylene from the ring eliminates affinity.

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