A basic residue C-terminal to tyrosine compromises its viability as a tyrosine kinase target

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Many regulatory enzymes are phosphorylated on specific residues, either by the action of protein kinases (PK) or through autophosphorylation, leading to an alteration in their kinetic properties. PK substrate specificity determinants are generally held to involve primary structural features located near the site of phosphorylation, providing evidence of a PK recognition motif. Synthetic peptides have therefore played an important role in the study of PK substrate specificity as well as in the measurement of PK activities in cell extracts.

One of the merits of using defined peptides as models of in vivo phosphorylation sites is the derived ability to elucidate complex kinetic phenomena such as multisite or sequential phosphorylation. Multiple autophosphorylation characterises the activation of receptor protein tyrosine kinases (PTKs). While autophosphorylation itself may well be a sterically and kinetically favourable reaction, reliant upon the organisation of the phosphorylation site(s) within the PTK substrate, our studies on substrate recognition by the human insulin receptor (hIR) PTK domain [1,2] provide evidence for a strongly deterministic role for the primary sequence context of the target tyrosine in auto- and substrate phosphorylation.

To investigate whether our conclusions regarding charge-related determinants of tyrosine target selectivity found for the hIRPTK extend to other receptor PTKs, we have gone on to study the phosphorylation efficacy of Tyr845 of the EGF receptor (EGFR). This tyrosine is located in the regulatory loop region of the kinase domain which is implicated in modulating both the specificity and mechanism of PK phosphorylation [3]. While this region of the hIRPTK contains three closely sequential tyrosines (Y-1158, 1162 & 1163) whose autophosphorylation is associated with enhanced TK activity [4], Tyr845 is not autophosphorylated. Rather, the four detectable sites of autophosphorylation occur in the EGFR kinase tail region [5].

Comparison of the regulatory loop sequences of the two receptor PTKs [6, Table 1] showed that both Tyr845 (EGFR) and Tyr1162 (hIR) are preceded by an acidic residue, commonly held to be a positive determinant for tyrosine target recognition and phosphorylation. On these grounds it was expected that the lack of autophosphorylation at Tyr845 may directly reflect steric impediments to regulatory loop phosphorylation of the EGFR.

Using proton NMR spectroscopy to visualise tyrosine phosphorylation kinetics in real time, we undertook a comparative study of peptides comprising the regulatory loop regions of the EGFR (EGF core peptide) and the hIR (YYY peptide) as substrates for the soluble, expressed hIRPTK.

Unlike the ready phosphorylation of Tyr1162 of the YYY peptide (rate \( \sim 100\mu\text{mol} \cdot \text{min}^{-1} \cdot \mu\text{mol}^{-1} \text{enzyme} \)), phosphate incorporation on Tyr845 of the EGF core peptide proceeded at a much slower rate indicating at least an eight-fold difference in \( K_{\text{M}} \) for the two substrates despite both tyrosines having an adjacent acidic residue prescribed as a positive selectivity determinant.

Since the YYY peptide is an effective inhibitor of autophosphorylation, not only for the hIRPTK but also for the EGFR and pp60 \(^{c-src} \) [7], this peptide must be able to interact similarly with these kinase core domains. The PTK substrate selectivity observed here between the EGFR core peptide and the hIR YYY peptide therefore directly reflect kinetic determinants of the PTK and are likely to be based on the sequence context of the target tyrosine residue.

In previous studies of tyrosine selectivity we have observed that a charged residue at position Y+1 down-regulates the effectiveness of the tyrosine as an potential substrate of the hIRPTK [2] with a greater deleterious effect on phosphorylation kinetics of a basic residue immediately C-terminal to the target tyrosine. Indeed, inspection of the regulatory loop sequences of PTKs [6] reveals that not only Tyr845 but also the potential tyrosine targets in these regions located immediately C-terminal to a basic residue (HK). The exceptions are the EGF receptor, Neu and Der kinases. Of the actual sites of autophosphorylation in the EGFR only one, Tyr1086, has a histidine at Y+1 position. This is a novel site in a C-terminally truncated receptor [5] therefore suggesting that increased accessibility also plays a role in the phosphorylation of this residue.

Thus our overall observations indicate not only that a basic residue C-terminal to tyrosine compromises its viability as a PTK target but also it is the sequence context of Tyr845 of the EGF receptor which underlies the lack of regulatory loop phosphorylation in the EGFR receptor subgroup of the PTK family. As a consequence, an alternative means of regulating these kinases is required.

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Table 1. Sequences of regulatory loop peptides

<table>
<thead>
<tr>
<th>Peptide &amp; residue n°</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>YYY</td>
<td>RRRDIYETDYRR</td>
</tr>
<tr>
<td>hIR 1155-1165</td>
<td>RRDIYETDYYRK</td>
</tr>
<tr>
<td>EGFR core</td>
<td>AEEKEYHAEG</td>
</tr>
<tr>
<td>EGF 840-849</td>
<td>AEEKEYHAEG</td>
</tr>
</tbody>
</table>

Tyrosine phosphorylated in each peptide is highlighted.