Selecting digestion of a membrane transport protein

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Recently we showed that, in the presence of KCl, trypsin digestion of vesicles derived from pigeon erythrocyte membranes inactivated Na⁺-dependent uptake of alanine by the vesicles, whereas digestion in the presence of NaCl did not (Charalambous & Wheeler, 1985). Considerable degradation of vesicle proteins occurred under both conditions. These observations were interpreted as evidence for a Na⁺-induced conformation change in the alanine transport protein that somehow protected it from trypsin digestion. Hence the question arose as to whether the protection afforded by Na⁺ ions was specific to the action of trypsin or whether digestion by other proteases would be affected similarly. We have, therefore, examined the effects of NaCl and KCl on the actions of three other proteases: papain, thermolysin and a-chymotrypsin.

Plasma membranes were prepared from pigeon erythrocytes by a slight modification of the method of Watts & Wheeler (1980b) and a vesicular suspension was obtained as described previously (Watts & Wheeler, 1980b). The vesicles were prepared in mannitol medium (300 mm-mannitol, 1 mm-MgSO₄, 5 mm-L-alanine, 5 mm-Tris/HCl, pH 7.6 at 20°C) containing 1 mm-[³H]sucrose to provide a means of measuring intravesicle volume. Samples of vesicles were incubated for 30 min at 37°C in either 100 mm-NaCl or 100 mm-KCl containing the protease at a final concentration of 0.1 mg/ml. Control samples were incubated similarly without the protease. The mixtures were then diluted with ten volumes of chilled mannitol medium, centrifuged at 16 000 × g for 15 min, and the vesicles resuspended to the original volume in mannitol medium. Samples of each suspension were used for assay of Na⁺-dependent uptake of alanine (Watts & Wheeler, 1980b), phospholipid and protein content (Watts & Wheeler, 1980b), and analysis of residual proteins by polyacrylamide-gel electrophoresis (Laemmli, 1970). Na⁺-dependent uptake of alanine was taken as the difference in uptakes measured in the absence and presence of 5 mm-L-serine in Na⁺-medium (Charalambous & Wheeler, 1985).

The results in Table I show that exposure of the membrane vesicles to the different proteases in the presence of KCl caused decreases in subsequently measured Na⁺-dependent uptake of alanine of 84–90%. In contrast, after treatment with the proteases in the presence of 100 mm-NaCl, the vesicles retained 66–78% of their Na⁺-dependent uptake of alanine. The protein content of the vesicles, expressed as mg of protein/µmol of phospholipid, decreased by an average of about 30% after exposure to the proteases, there being no significant differences in the extent of degradation occurring in the presence of NaCl compared with that in KCl. Similarly, analysis of the residual proteins by polyacrylamide-gel electrophoresis revealed no differences in the profiles resulting from the actions of the different proteases or different ionic conditions. The pattern was similar to that described previously for the action of trypsin (Charalambous & Wheeler, 1985), with four main protein bands of Mₐ about 22 000, 34 000, 42 000 and 50 000–60 000.

The overall similarity between the results described above and those described for digestion by trypsin (Charalambous & Wheeler, 1985) indicates that the protection by Na⁺ ions of the alanine-transport system from the action of proteases is not linked with the specificity of the proteases. Hence it appears that a part of the transport protein containing several amino acid residues must be effectively shielded from the proteases in the presence of Na⁺ ions, but exposed to them in the presence of K⁺ ions. It is possible that this part of the protein moves very close to, or perhaps actually becomes embedded in, the lipid bilayer in the presence of Na⁺ ions. It is also possible that this effect is not caused by Na⁺ ions alone but results from a co-operative action of Na⁺ ions and alanine, because the vesicles were prepared in, and contained, 5 mm-alanine. This is necessary because the transport system is essentially an exchange system (Watts & Wheeler, 1978; Wheeler, 1982). Although examination of this possibility presents technical problems, they probably can be overcome and further work should provide more insight into some of the structure–function relationships of the transport system, which may be typical of other Na⁺-dependent transport processes in membranes.

This work was supported by a grant from the Medical Research Council.


Table I. Differential effects of NaCl and KCl on protease actions

<table>
<thead>
<tr>
<th>Digestion in medium containing:</th>
<th>Na⁺-dependent uptake of alanine (% of control value)</th>
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<tbody>
<tr>
<td></td>
<td>NaCl</td>
</tr>
<tr>
<td>Papan</td>
<td>78 ± 6</td>
</tr>
<tr>
<td>Thermolysin</td>
<td>66</td>
</tr>
<tr>
<td>a-Chymotrypsin</td>
<td>77</td>
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