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Poster presentation

Nucleotidyl cyclase activity of recombinant soluble guanylyl cyclase

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Background

The ubiquitously expressed soluble guanylyl cyclase (sGC) converts guanosine 5'-triphosphate (GTP) to guanosine 3':5'-cyclic monophosphate (cGMP). The heterodimeric protein is activated by nitric oxide (NO). sGC plays a key role in the regulation of vascular tone and neurotransmission. Hence, sGC is an important target for the treatment of cardiovascular diseases e.g. pulmonary hypertension, heart failure and coronary heart disease. In addition to the biosynthesis of cGMP, we had previously shown by radiochemical analysis that sGC also generates the cyclic pyrimidine nucleotide uridine 3':5'-cyclic monophosphate (cUMP)[1]. However, cUMP could only be described as putative product but an exact identification by structure analysis was still missing. Therefore, we have established a new analytical method based on high performance liquid chromatography/mass spectrometry (HPLC-MS/MS) [see poster: CM Spangler et al.]. In comparison with classic radiochemical assays an analysis by HPLC-MS/MS allows the detection of non-radioactive compounds. Accordingly, a broader spectrum of substrates that is not available for radioactive assays can be used. Additionally, different nucleotides can be detected at the same time and the detection is directly structure-based. Making use of the new HPLC-MS/MS method we systematically characterised the substrate specificity of sGC.

Materials and methods

Highly purified recombinant sGC from rat (α1β1) was activated by sodium nitroprusside (100 μM). sGC (5 ng/tube) was incubated at 37°C with 3 mM $MnCl_2$ and 200 μM of various nucleoside 5'-triphosphates (NTPs). Samples were stopped by heating at 95°C after 20 minutes. Concentrations of adenosine 3':5'-cyclic monophosphate (cAMP), inosine 3':5'-cyclic monophosphate (cIMP), uridine 3':5'-cyclic monophosphate (cUMP), cytidine 3':5'-cyclic monophosphate (cCMP) and thymidine 3':5'-cyclic monophosphate (cTMP) were determined by HPLC-MS/MS.

Results

We could demonstrate that besides GTP, sGC converts adenosine 5'-triphosphate (ATP), inosine 5'-triphosphate (ITP) and uridine 5'-triphosphate (UTP) to their corresponding cyclic nucleoside 3':5'-cyclic monophosphates (cNMPs). In relation to cGMP-synthesis cAMP-, cIMP- and cUMP-production amounted to 8%, 26% and 3.5%, respectively. To this end, we have not detected cCMP or cTMP production. Cytosine 5' triphosphate (CTP) and thymidine 5'-triphosphate (TTP) will be further examined as potential sGC substrates following method optimization regarding incubation time and protein concentration.

Conclusion

Our results show that sGC has broader substrate specificity than previously assumed. We could demonstrate that not only bacterial toxins [see poster: M Göttle et al.] but also a mammalian enzyme of high physiological importance catalyses the biosynthesis of rare cNMPs such as cUMP and cIMP.

References

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