Regulated Changes in NO Sensitivity of an Fe-S Cluster: Role of S138 Phosphorylation in the Function of Iron Regulatory Protein 1 / Cytosolic Aconitase

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Aconitases are ubiquitous Fe-S enzymes functioning in intermediary metabolism and/or in post-transcriptional gene regulation. Inherent differences in the sensitivity of the Fe-S cluster of aconitases to disruption by oxygen radicals has been suggested to be important in their metabolic and regulatory roles. In higher eucaryotes, cytosolic aconitase (c-acon) plays a dual role both as an aconitase with a [4Fe-4S] cluster and as a high-affinity RNA binding protein (IRP1) when the Fe-S cluster is removed. The interconversion between c-acon and IRP1 is an iron-dependent means of regulating the synthesis of proteins involved in iron metabolism. In addition to iron, the interconversion between c-acon and IRP1 can be influenced by phosphorylation, oxidative stress and NO. We have previously observed that when S138, one of two phosphorylation sites in IRP1, is mutated to aspartate or glutamate to mimic the effects of phosphorylation, the Fe-S cluster becomes markedly more sensitive to disruption by oxygen or oxygen radicals. To further understand the role of S138 phosphorylation in controlling the function of IRP1/c-acon we investigated the effect of NO on the stability of the Fe-S cluster of WT and S138 phosphomimetic mutants. In purified IRP1, [4Fe-4S] cluster reconstitution produced comparable aconitase activity for WT and mutant IRP1 (S138A, S138D, S138E). Treatment with the NO donor DEANO resulted in 50% loss of aconitase activity in 2-6 minutes for S138D and S138E mutants, while WT and the S138A mutant had a half life greater than 4 h under the same experimental conditions. A dose-response study showed the most severe phenotype for S138E, which was 50% inactivated with a dose of 7 mol DEANO/mol c-acon. The dose required to inactivate S138D to the same degree was 36 mol DEANO/mol c-acon, while for WT and S138A it was 365 mol DEANO/mol c-acon. Citrate afforded protection from DEANO-induced loss of aconitase activity. Our results suggest that S138 phosphorylation alters the threshold for regulation of RNA binding activity of IRP1 in response to iron, oxidative stress and NO through regulated changes in Fe-S cluster stability. We suggest a model wherein the stability of the Fe-S cluster of cytosolic aconitase can be modulated in order to promote the protein's varied roles in cellular function. (Support: NIH DK47219)