

Characterization of a 2',5'-oligoadenylate (2-5A)-dependent 37-kDa RNase L: azido photoaffinity labeling and 2-5A-dependent activation.

Shetzline SE, Suhadolnik RJ. (2001)

Department of Biochemistry and the Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140, USA.

Upregulation of key components of the 2',5'-oligoadenylate (2-5A) synthetase/RNase L pathway has been identified in extracts of peripheral blood mononuclear cells from individuals with chronic fatigue [corrected] syndrome, including the presence of a low molecular weight form of RNase L. In this study, analysis of 2',5'-Oligoadenylate (2-5A) binding and activation of the 80- and 37-kDa forms of RNase L has been completed utilizing photolabeling/immunoprecipitation and affinity assays, respectively. Saturation of photolabeling of the 80- and the 37-kDa RNase L with the 2-5A azido photoprobe, [(32)P]pApAp(8-azidoA), was achieved. Half-maximal photoinsertion of [(32)P]pApAp(8-azidoA) occurred at 3.7×10^{-8} M for the 80-kDa RNase L and at 6.3×10^{-8} M for the 37-kDa RNase L. Competition experiments using 100-fold excess unlabeled 2-5A photoaffinity probe, pApAp(8-azidoA), and authentic 2-5A (p(3)A(3)) resulted in complete protection against photolabeling, demonstrating that [(32)P]pApAp(8-azidoA) binds specifically to the 2-5A-binding site of the 80- and 37-kDa RNase L. The rate of RNA hydrolysis by the 37-kDa RNase L was three times faster than the 80-kDa RNase L. The data obtained from these 2-5A binding and 2-5A-dependent activation studies demonstrate the utility of [(32)P]pApAp(8-azidoA) for the detection of the 37-kDa RNase L in peripheral blood mononuclear cell extracts.

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