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Ribonuclease Inhibitor from *Streptomyces aureofaciens*

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An existence of natural protein ribonuclease inhibitor was discovered in 1952, when in guinea pig liver homogenates a latent RNase activity was observed (Pirotte and Desreux 1952) Ribonuclease activity could be revealed after acidification of the extract as a result of dissociation of RNase inhibitor complex Since then it has been shown, that an occurrence of ribonuclease inhibitor seems to be widespread (Lee and Vallee 1993) It has been detected in various mammalian tissues, nonmammalian organisms, fungi and bacteria as its free form or in the complex with endogenous ribonucleases In spite of its unambiguous role in RNA metabolism, a definite function for this protein is still not know with certainty (Hofsteenge 1997, Lee and Vallee 1952) Though a number of results have been obtained following different aspects of ribonuclease inhibitor occurrence, no experiments focused on its biological function have been published Hofsteenge summarised

three hypotheses for biological role of mammalian inhibitors (Hofsteenge 1997) stressing that for each of them some observations argue against it 1/ inhibitor inhibits intracellular RNases which regulate level of different kind of RNA High levels of inhibitor cause RNA accumulation, low levels cause its degradation 2/ inhibitor is a safeguard against RNases that are destined for secretion, but inadvertently appear in cytoplasm 3/ inhibitor regulates the physiological action of RNases such as angiogenin Ribonuclease inhibitors from other animal sources have not been studied in such detail and they might be structurally different

The ribonuclease inhibitors from fungi and bacteria form entirely distinct family of proteins (Hofsteenge 1997) Until recently the only protein from *Bacillus amyloliquefaciens*, called barstar have been known and well studied (Hartley 1997) This inhibitor inhibits extracellular RNase from the same strain and it is assumed, that its role is to prevent ribonuclease being active prior secretion, which would be extremely harmful to the cell Now we have the other bacterial ribonuclease inhibitor, produced by sporogenic grampositive bacteria *Streptomyces aureofaciens*(Krajcikova *et al* 1998) Though this protein was discovered in 1982 (Jelokova *et al* 1982), we started to work intensively on its isolation and purification after successful structural studies of streptomycete ribonuclease (Ševcík and Zelinka 1986, Sevcik *et al* 1991, Sevcik *et al* 1993a, Sevcik *et al* 1993b Sevcik *et al* 1996) for a complex which is formed between RNase and its inhibitor In general, RNase - RNase inhibitor interaction belongs to the tightest protein - protein interactions and their complex is considered to be an ideal natural model for study protein recognition processes Dissociation constant of these complexes have been determined to be on the order of 10^{-14}-10^{-16} M for both mammalian and bacterial inhibitors (Hartley 1997, Lee and Valle 1993) Therefor we assumed that also inhibitor from *Streptomyces* would bind to RNase Sa very tightly However studying aspects of RNase - inhibitor mutual interactions demands to have a pure proteins in sufficient amounts, and a problem how to isolate and purify inhibitor to homogeneity had to be solved It took rather long time to do it The main reason of our difficulties raised from a very low level of the inhibitor in cells (less than 50 g from 1 l of cultivation medium) and its unsatisfactory stability during purification As it has been shown affinity column with immobilised ribonuclease was the best manner to obtained the pure protein, though in minimal amounts An existence two inhibitor named SaI14 and SaI20 that were finely identified in SDS PAGE after affinity chromatography, has not be expected In previous work only one protein with inhibitory activity has been described in *Streptomyces aureofaciens* (Jelokova *et al* 1982), and also from *Bacillus amyloliquaefaciens* only barstar have been isolated Though we have no explanation for this phenomenon up to present time, there is no doubt about their existence The alignment of sequences SaI14 and SaI20 that were partially determined from their N-termini with barstar revealed significant similarities, especially between SaI14 and barstar (Krajcikova *et al* 1998)

Because of extremely low amounts of pure proteins which we have got at the end of purification procedure in the next step it was necessary to isolate their genes and prepare a recombinant proteins We focused on SaI14 protein and the cloning of its gene and over expression in pET expression system has already been finished 1 l of culture can provide yields at least 200 mg of the recombinant inhibitor, that is sufficient amounts for functional and structural studies, that will be our next goal in near future Employment of three streptomycete ribonucleases Sa, Sa2, Sa3 and their two inhibitors SaI14 and SaI20, and others as they become available, together with barnase and barstar, expands the study of enzyme-inhibitor complexes and should help clarify details of protein-protein recognition

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X-Ray Analysis of Yeast Glucoamylases Prepared by Gene Engineering Techniques

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Key words: glucoamylases, *Saccharomycopsis fibuligera*, crystal structure

In recent years there has been considerable interest in glucoamylase [exo-1,4-α-D-glucosidase, 1,4-α-D-glucan glucohydrolase, EC 3 2 1 3] because of its industrial importance Glucoamylases from various microbial sources (namely from *Aspergillus* sp , *Rhizopus* sp , *Saccharomycopsis fibuligera*) are used extensively in industry in the production of glucose Unfortunately, wild type glucoamylases are not suited ideally to their industrial roles, having pH of optimum catalysis and thermostability that are too low In order to