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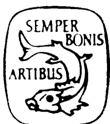

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Affinity Labelling of the Active Center of DNA-dependent RNA Polymerases within the Archaebacterial Kingdom

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Summary

The super-selective affinity labelling procedure for the active center of RNA polymerase from *Escherichia coli* (*Grachev* et al., 1987a) was successfully applied to the enzyme from archaebacteria. Using adenosine-5′-trimetaphosphate or the p-hydroxybenzaldehyde ester of ATP, the second largest subunit B′ of the RNA polymerase from methanogenic/halophilic and sulphate reducing archaebacteria, and the largest subunit B of the non-methanogenic thermophilic archaebacterium *Sulfolobus* sp. strain B 12 are labelled specifically. The labelling reaction is strictly template-dependent and blocked by the transcription inhibitor heparin. We present evidence that adenosine-5′-trimetaphosphate is attached close to the catalytic center of the RNA polymerase via a phosphoamide bond to lys or his residues.

The specific affinity labelling of subunit B′ from methanogens/halophiles together with the earlier observed immunological cross-reactivity (*Gropp* et al., 1986) indicates that this subunit as well as subunit B″ contains amino acid sequences which are homologous to sequences in subunit B of the enzyme from non-methanogenic thermophilic archaebacteria and in subunit β from *E. coli* RNA polymerase.

Key words: Archaebacteria – RNA polymerase – Catalytic center – Affinity labelling – Evolution

Introduction

The isolated RNA polymerases from archaebacteria have been shown to consist of 8–10 polypeptide chains (*Zillig* et al., 1985a). In their polypeptide pattern and in their antigenicity (*Huet* et al., 1983; *Gropp* et al., 1986) these enzymes resemble much more the eukaryotic RNA polymerases A, B and C than the RNA polymerase of *Escherichia coli.* To three of the four subunits β′, β, α and σ of the *E. coli* enzyme some functions could be assigned, e.g. to subunit β′ affinity to DNA, to subunit β the participation in rifampicin-binding and to subunit σ the promoter recognition (for review see *Yura* and *Ishihama,* 1979). In archaebacteria, the fifth largest polypeptide E of the *Halobacterium halobium* and of *Thermoplasma acidophilum* RNA polymerase has been reported to be required for transcription of natural DNA templates and to be dispensable for transcription of the synthetic template poly d(A–T) (*Madon* and *Zillig,* 1983; *Schnabel* et al., 1982). Up to now no further information is available on the role of the various polypeptides contained in purified archaebacterial RNA polymerases. The fact that the four largest components of these enzymes could be allied with each other by serological cross-reaction (*Schnabel* et al., 1983; *Thomm* et al., 1986) indicates that at least these polypeptides are necessary for the function of archaebacterial RNA polymerases. Immunological analyses revealed distinct differences in the RNA polymerase structure between different groups of archaebacteria (*Schnabel* et al., 1983). The RNA polymerases of methanogenic/halophilic archaebacteria contain two polypeptides B′ and B″, which both show serological cross-reaction to the largest subunit B of the enzymes from non-methanogenic thermophilic archaebacteria. According to this feature the AB′B″-C-type of methanogens/halophiles and the BAC-RNA polymerase type of non-methanogenic thermophiles can be distinguished (*Zillig* et al., 1985a). The RNA polymerase of the recently discovered sulphate reducing archaebacterium *"Archaeoglobus fulgidus"* (*Stetter* et al., 1987) harbours two polypeptides homologous to B′ and B″ of methanogens/halophiles. In contrast

Abbrevations: ATmP, Adenosine-5′-trimetaphosphate, ATP-A, 4-Hydroxybenzaldehyde ester of ATP

to all other archaebacteria containing this enzyme, the immunological determinants of the first (A) and fourth (C) component are present in one polypeptide ((A+C) B'B"-type; *Stetter* et al., 1987).

Recently, by "super-selective affinity labelling" the second largest subunit of the *E. coli* (*Grachev* et al., 1987a) and of the three RNA polymerases, A, B and C from yeast (*Riva* et al., 1987) have been shown to participate in the formation of the active center. Using the same method we identified in this study the subunit of three different types of archaebacterial RNA polymerases which carry the active center.

Materials and Methods

Purification of RNA polymerases

RNA polymerases of methanogenic bacteria, *Halobacterium* and *Sulfolobus* sp. strain B 12 were purified as decribed previously (for review see *Thomm* et al., 1986; *Zillig* et al., 1985a).

Synthesis of the reactive ATP derivatives

Adenosine-5'-trimetaphosphate (ATmP) and the p-hydroxybenzaldehyde ester of ATP (ATP-A) were synthesized as described by *Knorre* et al. (1976) and *Grachev* et al. (1987a), respectively.

Affinity labelling of RNA polymerases

Archaebacterial RNA polymerases were modified by cyclic adenosine-5'-trimetaphosphate (ATmP) or the p-hydroxybenzaldehyde ester of ATP (ATP-A) in a reaction mixture (10 µl) containing 11 mM HEPES, pH 7.9, 10 mM $MgCl_2$, and 2–5 µg RNA polymerase in the presence of the salt concentration optimal for each enzyme (*Thomm* et al., 1986; *Zillig* et al., 1985a). The final concentrations of the derivatives were 1.5 mM and 1 mM, respectively (5 min incubation at 37 °C. In experiments with ATP-A, 1 µl of a $NaBH_4$ solution (5 mg/ml) was added after the modification reaction to reduce the double-bond of the Schiff-base and the excess of the derivative, and incubation continued for 20 min at 0 °C. The modification with ATmP was performed for 25 min at 37 °C. After the addition of 1 µl poly d(A–T) (1 mg/ml) and 1 µl [α^{32}P]UTP (3000 Ci/mmol) a phosphodiester bond is formed between an enzyme-linked derivative close to the catalytic center and UTP during 20 min at the transcription temperature optimal for each enzyme (*Thomm* et al., 1986; *Zillig* et al., 1985a). To remove the radioactive RNA which could be formed by unmodified RNA polymerase the reaction mixture was treated with pancreatic RNase A (10 µg/ml; 15 min 37 °C). To degrade the template poly d(A–T), also pancreatic DNase (3 µg/ml), was added. After the RNase- and DNase-treatment the reaction was stopped with electrophoresis sample buffer as described previously (*Grachev* et al., 1986). The RNA polymerase subunits were separated electrophoretically in 5–25 percent exponential polyacrylamide gels in the presence of Na-dodecylsulfate (*Thomm* et al., 1986). The Coomassie-Blue stained gels were dried and labelled polypeptides identified by autoradiography at −70 °C intensified by Dupont Cronex Quanta II screen.

Isolation of the labelled dinucleotide ApU from the B subunit of Methanococcus vannielii

B' subunit from 20 µg *Mc. vannielii* RNA polymerase was isolated after affinity labelling with ATmP by electroelution from an unstained polyacrylamide gel employing a Biotrap BT 1000 apparatus (Schleicher & Schüll) (*Jacobs* and *Clad*, 1986). The eluted protein was precipitated with 4 volumes of acetone, dissolved in 180 µl H_2O and acidified to 50 mM HCl. After 1 h at 37 °C the pH was adjusted to 8.0 with tris-base, 2 units of calf intestinal alkaline phosphatase (Boehringer, Mannheim) were added and the mixture incubated 1 h at 37 °C. The solution was then dried *in vacuo* and the pellet redissolved in 5 µl of water. Comparison of the labelled compound with an ApU standard (Sigma, Taufkirchen) was performed by chromatography on polyethyleneiminecellulose-foils (Merck), Kieselgel 60 F_{254} thin layer plates (Merck) or Whatman 3 MM chromatography paper. The solvents used were 0.16 M LiCl or 0.5 M sodium formate pH 3.6 (PEI-cellulose), isopropanol : 25% NH_3 : H_2O = 6 : 3 : 1 (kieselgel thin layer plates) and 0.1 M sodium phosphate (pH 6.8) : $(NH_4)_2SO_4$: n-propanol = 100 : 60 : 2 (3 MM paper) (*Randerath* and *Randerath*, 1967; *Hanna* and *Meares*, 1983; *Bock* et al., 1956).

Results

Affinity labelling of RNA polymerases

In order to localize the active center on the subunits of archaebacterial RNA polymerases, the enzymes were modified with ATmP or ATP-A. After synthesis of labelled dinucleotides (*Grachev*, et al., 1986; see also Materials and Methods) the polypeptides of the RNA polymerases were separated by polyacrylamide gel electrophoresis in presence of dodecylsulphate and polypeptides containing covalently bound radioactive compounds identified by autoradiography. As a representative of the AB'B"C RNA polymerase type, the enzymes from *Mc. vannielii*, *Methanococcus thermolithotrophicus*, *Methanobacterium*

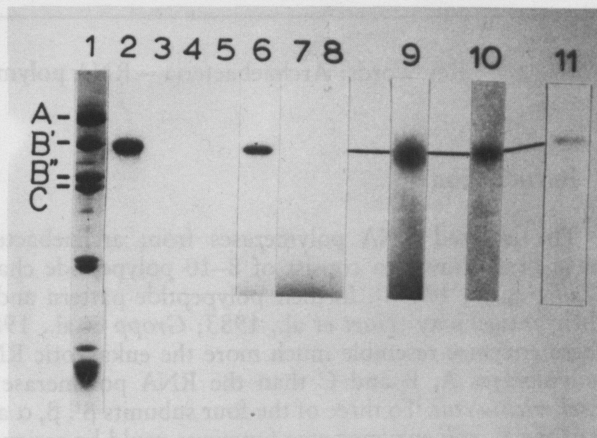

Fig. 1. Affinity labelling of the RNA polymerases from methanogenic/halophilic archaebacteria.
Polypeptides were separated after affinity labelling by SDS polyacrylamide gel electrophoresis and polypeptides associated with radioactivity identified by autoradiography. Lane 1, RNA polymerase of *Methanococcus vannielii* stained with Coomassie-Blue; lanes 2–8, autoradiogram of the affinity labelled *Mc. vannielii* RNA polymerase modified with ATP-A (lanes 2–5) and ATmP (lanes 6–8); lanes 3 and 7, without DNA template; lanes 4 and 8 in the presence of 100 µg heparin/ml (added immediately after the enzyme); lane 5, addition of $NaBH_4$ omitted; lane 9–11, RNA polymerase from *Mc. thermolithotrophicus*, *Methanobacterium thermoautotrophicum*, strain W, and *Halobacterium halobium* labelled with ATP-A as reactive nucleotide.

thermoautotrophicum, strain W and *Halobacterium halobium* were investigated. In the enzyme from *Mc. vannielii* only one labelled protein band was detected with both ATP-A and ATmP as reactive ATP analogue (Fig. 1, lanes 2 and 6). By comparison of the autoradiogram with the stained polyacrylamide gel (Fig. 1, lane 1), this subunit was identified as B′. In the absence of DNA (Fig. 1, lanes 3 and 7) and in the presence of the transcription inhibitor heparin (Fig. 1, lanes 4 and 8) no labelling occurred. These findings indicate that the modification of this subunit is dependent on conditions allowing initiation of RNA synthesis. As expected binding of ATP-A was unstable without addition of $NaBH_4$ and no radioactivity could be detected (Fig. 1, lane 5). With the enzymes from *Mc. thermolithotrophicus* and *Mb. thermoautotrophicum* W containing a subunit B′ of approximately the same size as *Mc. vaniellii*, the same polypeptide became labelled with ATP-A (Fig. 1, lanes 9 and 10) and with ATmP (data not shown). The same observation was made with the enzyme from *H. halobium*, where subunit B′ is about 8% larger than that of *Mc. thermolithotrophicus* (Fig. 1, lane 11).

In the archaebacterial sulphate-reducer (*Stetter* et al., 1987), which shows the (A+C)B′B″ RNA polymerase type, also subunit B′ was labelled both with ATP-A (Fig. 2, lanes 1 and 2) and ATmP (data not shown). The controls clearly show that also with this enzyme, labelling only occurs when the affinity reagent is bound to the polypeptide in a stable form (Fig. 2, lane 4) and when the conditions of a template dependent initiation of RNA synthesis are fulfilled (Fig. 2, lane 3). Again labelling is prevented by heparin (Fig. 2, lane 5).

To localize the active center on the polypeptides of the RNA polymerases from thermophilic non-methanogenic archaebacteria (BAC-type), the enzyme of *Sulfolobus* sp. strain B 12 was used for affinity labelling experiments. Treatment with ATmP (Fig. 3, lane 2) or ATP-A (Fig. 3, lane 5) resulted in specific labelling of the largest subunit B (Fig. 3, lane 1). The same was found with the enzyme from *Sulfolobus acidocaldarius* (data not shown). The corresponding controls (Fig. 3, lanes 3, 4 and 6–8) showed again that labelling was dependent on conditions appropriate for *in vitro* initiation of RNA synthesis.

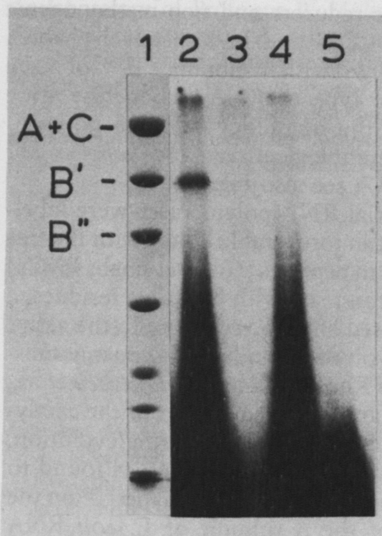

Fig. 2. Affinity labelling of the RNA polymerase from the archaebacterial sulfate reducer.
Lane 1, stained RNA polymerase (*Stetter*, et al., 1987; lane 2–5 autoradiography of enzyme labelled with ATP-A; lane 3 without DNA; lane 4 reduction step with $NaBH_4$ omitted; lane 5, in the presence of 100 μg heparin/ml.

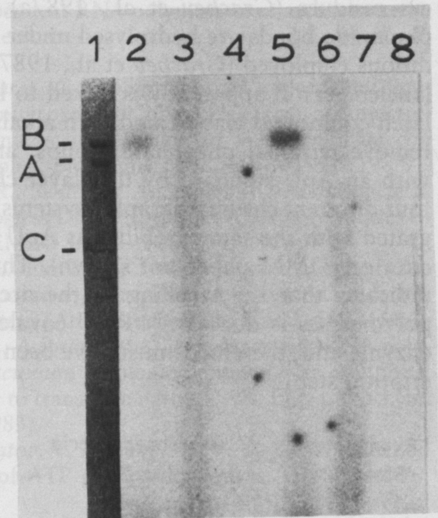

Fig. 3. Affinity labelling of the RNA polymerase from *Sulfolobus* sp. strain B 12.
Lane 1, stained RNA polymerase; lanes 2–4, autoradiographies of enzyme affinity labelled with ATmP, lanes 5–8 with ATP-A; lanes 3 and 6, without DNA; lanes 4 and 8, in the presence of 100 μg heparin/ml; lane 7, addition of $NaBH_4$ omitted.

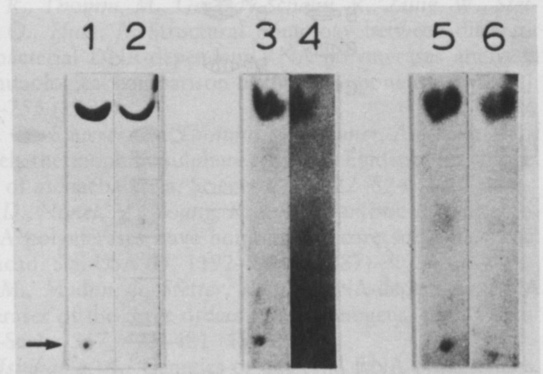

Fig. 4. Thin layer chromatography of the labelled product isolated from polypeptide B′ of *Mc. vannielii*.
The labelled compound released from the isolated subunit B′ of the *Mc. vannielii* RNA polymerase by mild HCl treatment was mixed with unlabelled ApU standard and subjected to chromatography on kieselgel thin-layer plates (lanes 1 and 2), and polyethyleneimine-cellulose (lanes 3–6) with sodium formate (lanes 3 and 4) and LiCl (lanes 5 and 6) as solvent. Unlabelled ApU was visualized by its UV fluorescence quenching (lanes 2, 4 and 6), the radioactive compound by autoradiography (lanes 1, 3 and 5). The arrow indicates the start.

To provide evidence that labelling of archaebacterial RNA polymerases occurs by covalently linked pppA[^{32}P]U we isolated the labelled compound attached covalently to the polypeptide. After affinity labelling with ATmP as the modifying derivative and [α-^{32}P]UTP, the B′ subunit of *Mc. vannielii* was isolated from a polyacrylamide gel. The labelled compound was released from the polypeptide by mild acid hydrolysis (50 mM HCl; 1 h 37 °C). In principle ATmP can form stable bonds with lys, his, ser, thr, tyr and cys residues (*Grachev* et al., 1987a). Since only phosphoamide bonds are hydrolysed under the mild acid conditions employed (*Grachev* et al., 1987a), the radioactive labeled ATmP apparently is linked to lys or his. The isolated compound was treated with alkaline phosphatase to remove terminal phosphate groups, and then compared with an ApU standard by thin-layer chromatography. In four different chromatographic systems the compound migrated with the same mobility as ApU (Fig. 4, lanes 1–6; data on 3 MM paper not shown). This finding strongly indicates that the labelling of the archaebacterial RNA polymerases is due to A[^{32}P]pU covalently bound to the enzyme and, therefore, must have been formed in a transcription step.

Fig. 5. Schematic representation of the subunit structure of the RNA polymerases from representatives of the three kingdoms of life.
Polypeptides which show serological cross-reaction are characterized by equivalent distinguishing marks. In the meantime, also subunit B from the yeast RNA polymerases has been shown to harbour the catalytic center (*Riva* et al., 1987). Note that subunit B of sulfur-dependent archaebacteria contains the determinants of B′ and B″ of methanogens and the sulphate reducer (*Stetter* et al., 1987). Polypeptides which have been shown to participate in the formation of the active center are indicated by an asterisk.

Discussion

The results reported above clearly demonstrate that the affinity labelling method for the active center of *E. coli* RNA polymerase developed by *Grachev* et al. (1987a) may also be applied to the archaebacterial RNA polymerases. The finding of a specific labelling of phage T7 RNA polymerase (*Grachev* et al., 1987c) consisting of a single polypeptide chain, of the three RNA polymerases A, B and C from yeast (*Riva* et al., 1987) and of wheat germ RNA polymerase B (*Grachev* et al., 1986) consisting of numerous polypeptides, suggests that this procedure can generally be applied for the identification of the polypeptides and a particular region thereof involved in the organisation of the active center of all DNA-dependent RNA polymerases. Its convenient application and its high specificity recommends it for comparative studies in systematics and evolution. Interestingly, the enzymatic activity of all RNA polymerases studied so far survived the covalent attachment of the affinity label at or close to the active center and also the rather harsh treatment of the enzyme with $NaBH_4$ for the reduction of the Schiff-base. Otherwise the labelling would not occur since it requires the enzymatic activity for the formation of the phosphodiester bond. Similar to eukaryotic RNA polymerases (*Grachev* et al., 1986; *Riva* et al., 1987) archaebacterial RNA polymerases become labelled by this procedure at only a single polypeptide. This is in contrast to the labelling of some eubacterial RNA polymerases such as the enzyme from *E. coli* where in addition to subunit β subunit sigma also becomes labelled although only weakly (*Grachev* et al., 1987a).

The specific labelling of the B′ subunit of RNA polymerases from four representatives of the methanogenic/halophilic type of enzyme clearly indicates that this subunit forms an essential part of the active center. In the archaebacterial sulphate-reducers and non-methanogenic thermophiles the subunits B′ and B, respectively, which cross-react serologically with subunit B′ of the methanogenic/halophilic type of RNA polymerase were labelled, corroborating the homology of these polypeptides inferred from immunological data (*Schnabel* et al., 1983; *Stetter* et al., 1987; see also Fig. 5).

Since all archaebacterial RNA polymerases were labelled with ATP-A, which can form stable bonds with the free ε-amino group of lysine in peptides (*Grachev* et al., 1987a) and with ATmP which can react with lys or his residues, a lys residue must be located at least very close to the active center. Lysine is also involved in binding of the same substrate analogue to *E. coli* RNA polymerase (*Grachev* et al., 1987a). Therefore, this amino acid as a part of the catalytic center, seems to be highly conserved during evolution. Recently the attached labelled dinucleotide was found to be localized within the region from ile^{1036} to met^{1066} on the amino acid sequence of the β subunit of *E. coli* RNA polymerase (*Grachev* et al., 1987b). This region contains an amino acid sequence (from leu^{1047} to arg^{1058}) which is strongly homologous to the region from leu^{961} to lys^{972} in the second largest subunit of yeast RNA polymerase B (*Sweetser* et al., 1987). It is this subunit which becomes labelled when the method of affinity labelling used here is

applied to the yeast B enzyme (*Riva* et al., 1987). When inspecting these homologous amino acid sequences (*Sweetser* et al., 1987) it becomes evident that they can be folded into an α-helix which is rich in basic amino acids on one side, a property which should be an advantage for the binding of negatively charged nucleotide residues in the active center of RNA polymerases. We would expect the conservation of homologous regions also in the B′ or B subunits of archaebacteria. Indeed, subunit B′ of the methanogenic/halophilic enzyme which participates in the formation of the active center (Fig. 1) serologically cross-reacts with the second largest subunit of eukaryotic RNA polymerases (*Gropp* et al., 1986; see schematic representation in Fig. 5) indicating an homology in functional and antigenic structure with the second largest subunit of eukaryotic RNA polymerases.

Since the archaebacterial subunit B″ also shows homology with subunit β from *E. coli* as is indicated by serological cross-reaction (*Gropp* et al., 1986; Fig. 5) both subunits B′ and B″ obviously correspond to different parts of subunit β from *E. coli*. Our findings are in contrast to the suggestion that the eubacterial subunit β may have evolved from an RNA polymerase of the AB′B″ C type by the loss of subunit B′ (*Zillig* et al., 1985b). The subunit B of non-methanogenic thermophiles and β from *E. coli* RNA polymerase share the feature that they contain important regions of B′ and B″ in one polypeptide. This may be considered as an indication that eubacterial RNA polymerases are phylogenetically more related to the enzymes from non-methanogenic thermophiles than to those from methanogens/halophiles.

Acknowledgement. Highly purified RNA polymerase preparations from *Halobacterium halobium* and *Sulfolobus* sp. strain B 12 were generously provided by *F. Gropp* and *W.-D. Reiter* from the laboratory of Professor *W. Zillig*, Max-Planck-Institut für Biochemie, Martinsried. A. J. L. wishes to thank *F. Gropp* and *W.-D. Reiter* for helpful advice for the assay and purification of these enzymes. The investigations of A. J. L. and G. R. H. were supported by the Deutsche Forschungsgemeinschaft (SFB 304) and the Fonds der Chemischen Industrie, the studies of M. T. and K. O. S. by grants of the Deutsche Forschungsgemeinschaft.

References

Bock, R. M., Ling, S. N., Morell, S. A., Lipton, S. M.: Ultraviolett absorption spectra of adenosine-5′-triphosphate and related 5′-ribonucleotides. Arch. Biochem. Biophys. 62, 253–264 (1956)

Grachev, M. A., Hartmann, G. R., Maximova, T. G. Mustaev, A. A., Schäffner, A. R., Sieber, H., Zaychikov, E. F.: Highly selective affinity labelling of RNA polymerase B (II) from wheat germ. FEBS Lett. *200,* 287–290 (1986)

Grachev, M. A., Kolocheva, T. I., Lukhtanov, E. A., Mustaev, A. A.: Studies on the functional topography of the *Escherichia coli* RNA polymerase. Highly selective affinity labelling by analogous of initiation substrates. Eur. J. Biochem. *163,* 113–121 (1987a)

Grachev, M. A., Lukhtanov, E. A., Mustaev, A. A., Richter, V. A., Rabinov, I. V., Skoblov, Yu. S., Abdukayumov, N. N.: Localisation of the lysine residues nearby the site of initiating substrate binding of *E. coli* RNA polymerase. Bioorg. Khim. *13,* 552–555 (1987b)

Grachev, M. A., Zaychikov, E. F., Lukhtanov, E. A., Maximova, T. G., Mustaev, A. A.: Highly selective affinity labelling of T7 phage RNA polymerase. Bioorg. Khim. *13,* 568–570 (1987c)

Gropp, F., Reiter, W. D., Sentenac, A., Zillig, W., Schnabel, R., Thomm, M., Stetter, K. O., Huet, J.: Homologies of components of DNA-dependent RNA polymerases of archaebacteria, eukaryotes and eubacteria. System. Appl. Microbiol. *7,* 95–101 (1986)

Hanna, M. M., Meares, C. F.: Synthesis of a cleavable dinucleotide photoaffinity probe of ribonucleic acid polymerase: application to trinucleotide labeling of an *Escherichia coli* transcription complex. Biochemistry 22, 3546–3551 (1983)

Huet, J., Schnabel, R., Sentenac, A., Zillig, W.: Archaebacteria and eukaryotes possess DNA-dependent RNA polymerases of a common type. EMBO J. 2, 1291–1294 (1983)

Jacobs, E., Glad, C.: Elution of fixed and stained membrane proteins from preparative dodecyl sulfate-polyacrylamide gels into a membrane trap. Anal. Biochem. *154,* 583–589 (1986)

Madon, J., Zillig, W.: A form of the DNA-dependent RNA polymerase of *Halobacterium halobium* containing an additional component is able to transcribe native DNA. Eur. J. Biochem. *133,* 471–474 (1983)

Knorre, D. G., Kurbatov, V. A., Samikov, V. V.: General method for the synthesis of ATP gamma-derivatives. FEBS Lett. *70,* 105–108 (1976)

Randerath, K., Randerath, E.: Thin-layer separation methods for nucleic acid derivatives. Meth. Enzymol. *12A,* 323–347 (1967)

Riva, M., Schäffner, A. R., Sentenac, A., Hartmann, G. R., Mustaev, A. A., Zaychikov, E. F., Grachev, M. A.: Active site labelling of RNA polymerases A, B and C from yeast. J. Biol. Chem. *262,* 14377–14380 (1987)

Schnabel, R., Zillig, W., Schnabel, H.: Component E of the DNA-dependent RNA polymerase of the archaebacterium *Thermoplasma acidophilum* is required for the transcription of native DNA. Eur. J. Biochem. *129,* 473–477 (1982)

Schnabel, R., Thomm, M., Gerardy-Schahn, R., Zillig, W., Stetter, K. O., Huet, J.: Structural homology between different archaebacterial DNA-dependent RNA polymerases analyzed by immunological comparison of their components. EMBO J. *2,* 751–755 (1983)

Stetter, K. O., Lauerer, G., Thomm, M., Neuner, A.: Isolation of extremely thermophilic sulphate reducers. Evidence for a novel branch of archaebacteria. Science *236,* 822–824 (1987)

Sweetser, D., Nonet, M., Young, R. A.: Prokaryotic and eukaryotic RNA polymerases have homologous core subunits. Proc. Natl. Acad. Sci. USA *84,* 1192–1196 (1987)

Thomm, M., Madon, J., Stetter, K. O.: DNA-dependent RNA polymerases of the three orders of methanogens. Biol. Chem. Hoppe-Seyler *367,* 473–481 (1986)

Yura, T. Ishihama, A.: Genetics of bacterial RNA polymerases. Annu. Rev. Genet. *13,* 59–97 (1979)

Zillig, W., Stetter, K. O., Schnabel, R., Thomm, M.: DNA-dependent RNA polymerases of the archaebacteria. In: The Bacteria, Vol. VIII, Archaebacteria (*C. R. Woese, R. S. Wolfe,* eds.), pp. 499–521. New York, Academic Press 1985a

Zillig, W., Schnabel, R., Stetter, K. O., Thomm, M., Gropp, F., Reiter, W. D.: The evolution of the transcription apparatus. In: Evolution of prokaryotes (*K. H. Schleifer, E. Stackebrandt,* eds.), pp. 45–71. New York, Academic Press 1985b

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A Comparative Study of the Amylolytic Ability of *Lipomyces* and *Schwanniomyces* Yeast Species

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Summary

The starch degrading ability of the yeast genera *Lipomyces* and *Schwanniomyces* were evaluated on solid and in liquid media. Strains of *Lipomyces kononenkoae, L. starkeyi* and *Schwanniomyces occidentalis* utilized 100% of the starch supplied, with biomass yield coefficients of up to 0.52. There was little correlation between the clearing zone diameters on starch agar plates and the extracellular amylase activities. A derepressed mutant strain of *L. kononenkoae* had the highest α-amylase activity (more than three-fold higher than the other yeasts) and glucoamylase activity (more than five-fold higher), while *L. starkeyi* exhibited the highest debranching activity. Furthermore, the *L. kononenkoae* mutant was the only yeast insensitive to glucose repression of its amylases.

Key words: *Lipomyces* – *Schwanniomyces* – Starch – Amylolytic yeast – Amylase – α-Amylase – Glucoamylase – Pullulanase

Introduction

Of the approximately 400 yeast species currently recognized, about a hundred are capable of utilizing starch as carbon and energy source (*Spencer-Martins* and *Van Uden*, 1977). Most of the amylolytic yeasts are unable to totally hydrolyze starch, however. Only a few species, therefore, show any promise for the direct bioconversion of starch to single cell protein.

Of the considerable research in recent years on starch degrading yeasts and on the bioconversion of starchy substrates to ethanol or yeast protein, relatively few studies were concerned with the differential biosynthesis of α-amylase, glucoamylase and debranching enzymes. A few yeasts have been reported to possess all three these enzymes, including *Saccharomycopsis fibuligera* (*Lemmel* et al., 1980; *Touzi* et al., 1982; *Ueda* and *Saha*, 1982; *De Mot* et al., 1984), *Lipomyces kononenkoae* (*Van Uden* et al., 1980; *Sá-Correia* and *Van Uden*, 1981; *Estrela* et al., 1982; *De Mot* et al., 1984), *Lipomyces starkeyi* (*Touzi* et al., 1982; *De Mot* et al., 1984; *Kelly* et al., 1985) and *Schwanniomyces occidentalis* (*Oteng-Gyang* et al., 1980; *Wilson* and *Ingledew*, 1982; *Touzi* et al., 1982; *Sills* and *Stewart*, 1982; *De Mot* et al., 1984).

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The data presented in the literature do not permit a direct comparison of the starch degrading capacity of amylolytic yeasts because (i) several different amylase assays are in use, (ii) different parameters for amylolytic activity have been employed, (iii) few reports include data on all three types of amylolytic enzymes, and (iv) different media and cultivation conditions have been used. In this investigation the amylase activities, glucose catabolite repression of the amylases, the effect of growth temperature on starch hydrolysis and the growth parameters of amylolytic yeasts, selected on the basis of the published values of their amylase activities and biomass yields (*Spencer-Martins* and *Van Uden*, 1977; *Spencer-Martins* and *Van Uden*, 1979; *Sills* and *Stewart*, 1982; *Dhawale* and *Ingledew*, 1983; *De Mot* et al., 1984), were quantitated under standardized conditions to facilitate a comparative evaluation.

Materials and Methods

Microorganisms. The strains of *Lipomyces* and *Schwanniomyces* used are listed in Table 1. Those denoted by the code IGC were kindly supplied by Prof. *N. van Uden*, Gulbenkian Institute