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The Glucose Effect and Regulation of α-Amylase Synthesis in the Hyperthermophilic Archaeon *Sulfolobus solfataricus*

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An α-amylase was purified from culture supernatants of *Sulfolobus solfataricus* 98/2 during growth on starch as the sole carbon and energy source. The enzyme is a homodimer with a subunit mass of 120 kDa. It catalyzes the hydrolysis of starch, dextrin, and α-cyclodextrin with similar efficiencies. Addition of exogenous glucose represses production of α-amylase, demonstrating that a classical glucose effect is operative in this organism. Synthesis of [^{35}S]-α-amylase protein is also subject to the glucose effect. α-Amylase is constitutively produced at low levels but can be induced further by starch addition. The absolute levels of α-amylase detected in culture supernatants varied greatly with the type of sole carbon source used to support growth. Aspartate was identified as the most repressing sole carbon source for α-amylase production, while glutamate was the most derepressing. The pattern of regulation of α-amylase production seen in this organism indicates that a catabolite repression-like system is present in a member of the archaea.

Catabolite repression is a paradigm for studies concerned with global and specific gene control mechanisms (22). In prokaryotes, catabolite repression together with transient repression and inducer exclusion make up what has been termed the glucose effect or repression of catabolic enzyme synthesis by glucose (23). However, for eukaryotes, the term catabolite repression is more generally used as a pseudonym for the glucose effect. In fact, evidence for transient repression, inducer exclusion, and requisite aspects of catabolite repression, including the ability to grow most rapidly on preferred carbon sources, is not well demonstrated (for reviews, see references 29 and 31). Catabolite repression in prokaryotes and eukaryotes has received wide attention, but the existence of an analogous process in the archaea has not been addressed. One hallmark of this process in gram-negative bacteria consists of the global mode of gene regulation of catabolite-repressible genes mediated by the small molecule cyclic AMP (cAMP) (8). Although the role of cAMP in some prokaryotes is well accepted, it has been eliminated as an effector in the corresponding catabolite response in the gram-positive bacterium *Bacillus subtilis* (for a review, see reference 14). In eukaryotes, including the budding yeast *Saccharomyces cerevisiae*, cAMP plays a crucial but indirect role in mediating the glucose effect. Interestingly, cAMP has been reported in a range of archaea (21).

Sulfolobus solfataricus is an extremely thermophilic organism which inhabits acidic hot springs. *S. solfataricus* is a member of the archaea and has been assigned to a subdivision termed the crenarchaeota by rRNA gene (rDNA) sequence analysis (32). It is capable of diverse modes of metabolism at temperatures ranging between 70 and 90°C. It can grow either lithoautotrophically, oxidizing sulfur (4, 15), or chemoheterotrophically on starch or other sugars as sole carbon and energy sources (7, 11). Recent studies also suggest that hot springs contain a previously unrecognized diversity of thermophilic archaea (2).

In many organisms, the catabolism of starch depends on a secreted α-amylase which generates linear maltodextrins from starch as well as a cell-associated α-glucosidase (maltase) which converts maltose and maltodextrins to glucose (16). α-Amylases (5, 17, 19, 20) and an α-glucosidase (6) have been characterized in the hyperthermophilic archaeon *Pyrococcus furiosus* (9). This organism is an obligate anaerobe and has an essential requirement for peptides (9, 30). It has been classified as a member of the other major subdivision of the archaea termed the euryarchaeota (32).

Members of the aerobic genus *Sulfolobus* can utilize starch as the sole carbon and energy source (11) and have both α-amylase and α-glucosidase activities (3). We recently reported the purification and characterization of a soluble maltase (α-glucosidase) from *S. solfataricus* 98/2 (28). The studies reported here were undertaken to better characterize the regulation of starch assimilation in this hyperthermophilic crenarchaeote. A secreted α-amylase was purified and its production was characterized during heterotrophic growth on a range of carbon sources. The observed pattern of α-amylase synthesis indicates that a catabolite repression-like system is operative in *S. solfataricus.*

MATERIALS AND METHODS

Strains and cultivation. The identity of *S. solfataricus* 98/2 was confirmed by rDNA sequence analysis of PCR-cloned rDNA fragments derived from clonal populations of this strain (13) and from *S. solfataricus* P2 (34). GenBank accession numbers for the resulting rDNA sequences are L36990 (strain 98/2) and L36991 (strain P2). Comparison of these sequences with previously published citations for *S. shibatae* (10 [GenBank accession number M32504]) and *S. acidocaldarius* (18, 25, 33 [GenBank accession number X03235]) confirm the identity of strain 98/2 as *S. solfataricus* (28).

Cultures were grown in screw-cap flasks at 80°C with shaking. The defined minimal medium was as described elsewhere (1), as modified previously (4), at a pH of 3.0. Carbon source supplements to basal salts were prepared as concentrated filter-sterilized stock solutions and were added after sterilization of the minimal medium. Glucose, dextrin, and starch were added at final concentrations of 0.2% (wt/vol). Aspartate and glutamate were added to final concentrations of 5 mM. Succinate was added to a final concentration of 17 mM, and all other carbon sources were added to final concentrations of 10 mM. Inocula for batch culture experiments used cells in the exponential phase of growth which were washed in minimal salts medium prior to inoculation. Growth was monitored spectrophotometrically at a wavelength of 540 nm. Cell densities were determined by direct microscopic examination under bright field, using phase optics. Soluble starch was from Fluka and was composed of both amylose and amylopectin. All other chemicals used were reagent grade.

Assays for α-amylase activity. α-Amylase activity was determined with an assay for the production of sugar reducing ends (26) or with an assay for the loss

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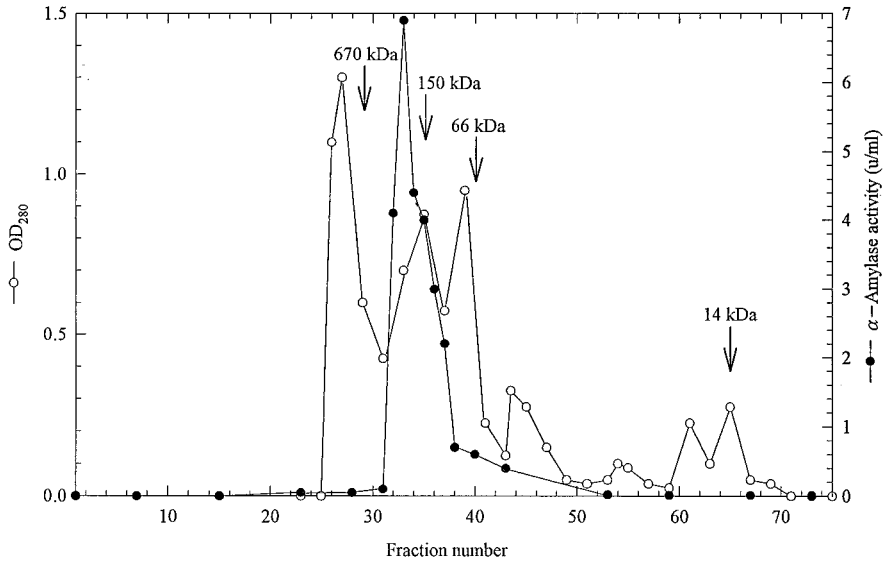

FIG. 1. Gel filtration chromatography of amylase activity. Concentrated culture supernatants were fractionated by FPLC. Samples were collected in 0.5-ml volumes and analyzed for α-amylase activity. OD_{280}, optical density at 280 nm.

of iodine binding to starch and dextrin (20, 24). Culture supernatants were prepared for enzyme assays by centrifuging 1-ml culture volumes at 5,000 × *g* for 20 min to remove the cells. The reducing-end assay was performed by adjusting the samples to 25 mM sodium acetate (pH 3.0), 0.5 mM calcium chloride, and 0.5% (wt/vol) starch and incubating them for 30 min at 80°C. The reaction was terminated by adjusting the sample to 0.04 M sodium carbonate. The sample was then adjusted to 15.4 mM potassium cyanide, 0.24 mM potassium ferricyanide, and 72.3 mM sodium carbonate and heated at 100°C for 10 min. The sample absorbance was determined at a wavelength of 420 nm and was corrected by subtracting the absorbance of a sample lacking added substrate. One unit of activity was defined as the amount of protein which produced 1 μmol of reducing ends in 1 min. Glucose was used as a reducing-end standard. The iodine binding assay was performed by combining 0.025 ml of clarified culture supernatant with 2% (wt/vol) starch and 100 mM sodium acetate (pH 3.0) at 80°C for 30 min. The reaction was terminated by cooling at 4°C. Color was developed by addition of 0.015 ml of an iodine solution (4% [wt/vol] potassium iodide, 1.25% [wt/vol] iodine). The sample absorbance was determined at a wavelength of 600 nm and was corrected for a sample lacking added substrate. One unit of activity was equivalent to the amount of protein which hydrolyzed 1 mg of starch in 1 min. For determination of the enzymatic activity of the purified α-amylase, the reducing-end assay was used. The iodine binding assay was routinely used to assay the α-amylase from culture supernatants unless otherwise indicated. Low levels of α-amylase were determined by using concentrated culture supernatant. Supernatant was harvested from cultures as indicated for routine assays but was then subjected to ultrafiltration in an Amicon Centricon concentrator with a 10,000-molecular-weight (MW) cutoff filter. Sample activities typically varied by less than 10%.

Protein purification. *S. solfataricus* was grown in basal salts medium with 0.2% starch as the carbon source in 0.5-liter volumes with shaking to a cell density of 5.6×10^9 cells per ml. All subsequent manipulations were carried out at 4°C. The supernatant from 5 liters of culture was clarified by centrifugation at 5,000 × *g* for 20 min and then concentrated by ultrafiltration to a final volume of 0.5 ml. Ultrafiltration was done in an Amicon concentrating unit with a Diaflo YM3 ultrafiltration membrane (3,000-MW cutoff) and a Centricon concentrator (10,000-MW cutoff). The concentrated supernatant was further clarified by ultracentrifugation at 208,000 × *g* for 20 min and then passed through a Spin-X centrifuge filter unit (CoStar) with a 0.22-μm-diameter cellulose acetate filter. The sample was then subjected to gel filtration fast protein liquid chromatography (FPLC), using a Superdex 75 H/R 10/30 (Pharmacia) column. The column was developed by using 100 mM sodium phosphate (pH 6.0). Standards used to estimate protein mass were porcine thyroglobulin (670 kDa), dog immunoglobulin G (150 kDa), bovine serum albumin (66 kDa), and hen egg white lysozyme (14 kDa). Fractions were assayed for activity by using the iodine binding assay. Protein concentrations were determined by the bicinchoninic acid assay (Pierce), and bovine serum albumin was used as a protein standard.

In vivo labeling and protein gel chromatography. Metabolic labeling was performed by using Trans-^{35}S-label (ICN) with a specific activity of 1,175 Ci/mmol. Cells were labeled in 10-ml amounts at a concentration of 10 μCi/ml for 2 h and then chased with a 1,000-fold molar excess of cold methionine for an additional 2 h. Samples were then chilled and centrifuged at 5,000 × *g* for 20 min to remove the cells. The supernatant was then adjusted to pH 6.9 and treated with *Bacillus* α-amylase (Sigma) at a concentration of 1 μg/ml for 30 min at 25°C. The sample was then adjusted to pH 4.5 and treated with *Rhizopus* glucoamylase (Sigma) at a concentration of 1 μg/ml for 1 h at 55°C. Following enzymatic treatment, the sample was concentrated by ultrafiltration and frozen at −20°C. Proteins were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using low-MW markers (Bio-Rad) as described previously (27). Autoradiograms were prepared by using X-Omat AR film (Kodak).

RESULTS

Purification of an α-amylase. *S. solfataricus* 98/2 was tested for the ability to grow on a defined minimal medium containing starch as the sole carbon and energy source. Growth was observed, and cell densities of 7×10^9/ml were achieved with 0.2% (wt/vol) starch. Spontaneous starch hydrolysis was not observed in uninoculated cultures. To determine whether the observed growth was due to a cell-associated or a cell-free starch degrading activity, sonicated cell pellets and the corresponding clarified culture supernatant were prepared and examined for relative levels of amylolytic activity. Maximum levels of activity detected during exponential phase growth were 0.125 (±0.003) U/mg of total protein in sonicated cell extracts and 1.7 (±0.003) U/ml in the corresponding sample of the culture supernatant. This result indicates that the bulk of the amylolytic activity was present in the culture in a cell-free form. To identify the protein(s) responsible for the observed amylolytic activity, the culture supernatant was collected and concentrated by ultrafiltration and then subjected to gel filtration chromatography. Amylolytic activity was confined to a single peak and exhibited an apparent mass of 240 kDa (Fig. 1). Active fractions were pooled and subjected to SDS-PAGE (Fig. 2). A protein with an apparent mass of 120 kDa was purified to near homogeneity (98.5%) by this procedure. This result suggests that the native form of the enzyme is a homodimer. To further investigate the nature of this amylolytic protein, its substrate utilization was examined at 80°C. Specific activities of the purified protein with starch, dextrin, and α-cyclodextrin as substrates were 340, 261, and 333 U/mg, respectively, and are within twofold of previously reported specific activities for other thermophilic α-amylases (17). The utilization of α-cyclodextrin indicates that this enzyme is capable of

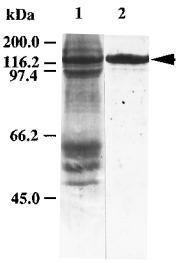

FIG. 2. SDS-PAGE of the purified α-amylase. Active gel filtration fractions were pooled and concentrated. Samples were then subjected to SDS-PAGE (12.5% [wt/vol] acrylamide gels), and gels were stained with Coomassie blue R250. The samples loaded were concentrated culture supernatant (lane 1, 100 μg of protein) and concentrated active FPLC fractions (lane 2, 20 μg of protein).

attacking a circularized substrate and therefore must have an endo-attack mechanism. The purified protein did not utilize β-cyclodextrin as a substrate, thus eliminating its assignment as a neopullulanase or amylopullulanase. This finding identifies the major secreted amylolytic activity of *S. solfataricus* 98/2 as an α-amylase, EC 3.2.1.1.

Glucose repression of α-amylase activity. Production of the α-amylase was significantly greater during growth on starch than during growth on glucose. Maximal α-amylase activities achieved were 1.7 (±0.003) and 0.13 (±0.004) U/ml, respectively, indicating that enzyme production was responsive to starch availability and varied over a 10-fold range. α-Amylase activity increased rapidly in culture supernatants when cells were subcultured from a glucose minimal medium to a starch minimal medium. Enzyme activity plateaued shortly after transfer to the starch-containing medium, and no further increase in secreted activity was observed for the duration of the batch culture growth cycle. Growth rates and cell yields were similar for the two cultures, indicating that these carbon sources were readily assimilated. The apparent plateau in the level of secreted α-amylase activity could reflect a balance between ongoing synthesis coupled with enzyme inactivation due to the harsh nature of the extracellular environment. To examine this possibility, a sample of cell-free supernatant containing α-amylase activity was subjected to prolonged incubation at 80°C at pH 3. After an additional 50-h incubation, the supernatant retained most (97%) of its starting activity. This result indicates that the plateau in secreted activity is unlikely to result from enzyme inactivation but rather results from the termination of enzyme production.

The termination of α-amylase production observed during growth on starch might reflect the accumulation of glucose as a repressing metabolite for α-amylase production. To explore this possibility, five *S. solfataricus* batch cultures, each containing an excess of starch at 0.2% (wt/vol) and glucose in amounts ranging from none to 0.1% (wt/vol), were examined for the appearance of α-amylase in the culture supernatant (Fig. 3). Presence of excess glucose (0.1% [wt/vol]) prevented α-amylase appearance altogether, while glucose omission resulted in immediate production of α-amylase following subculturing of the cells. Growth on a series of decreasing amounts of limiting glucose concentrations resulted in the appearance of α-amylase activity in the culture supernatant at increasingly early times during the growth cycle and corresponded to the cell densities observed for glucose exhaustion (Fig. 3). The culture density at which glucose was exhausted was determined in separate experiments in which these same concentrations of glucose were used as the sole carbon source. These glucose concentrations supported the following cell yields: 0.0125%,

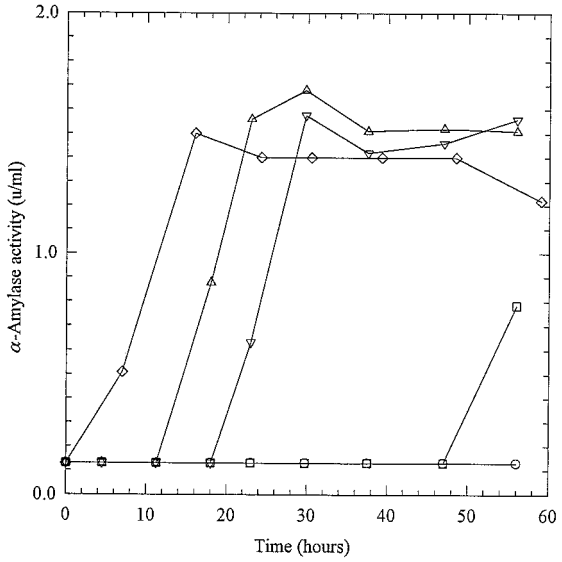

FIG. 3. Glucose repression of α-amylase production. Cells were grown in separate cultures with excess starch plus 0.1% (wt/vol) (circles), 0.05% (wt/vol) (squares), 0.025% (wt/vol) (inverted triangles), and 0.0125% (wt/vol) (triangles) glucose and with no added glucose (diamonds).

1.75×10^9 cells per ml; 0.025%, 3.0×10^9 cells per ml; and 0.05%, 5.3×10^9 cells per ml. Cell growth rates were largely unaffected during growth in the presence of excess starch and limiting glucose despite the requisite transition between growth on glucose and growth on starch. In addition, no induction of α-amylase activity was observed either in response to glucose starvation or in response to glucose resupplementation following glucose starvation.

To confirm that glucose was preventing α-amylase gene expression and not merely secretion of preformed enzyme, metabolic labeling was used to monitor synthesis of the enzyme de novo. Cells were grown in one culture in the presence of excess starch at 0.2% (wt/vol) and limiting glucose at 0.025% (wt/vol). Culture samples were labeled prior to the appearance of α-amylase in the culture supernatant and concomitantly with the appearance of secreted enzyme activity (Fig. 4A). In a second culture, cells were labeled during growth in the presence of excess starch and glucose. The sample supernatants from both cultures were then recovered and treated to remove residual polysaccharide which would otherwise obscure sample analysis. The treated samples were then concentrated by ultrafiltration and subjected to SDS-PAGE. An abundant labeled protein with an apparent mass of 120 kDa, identical to that of the purified α-amylase monomer, was observed in supernatant samples concomitantly with the appearance of secreted α-amylase activity (Fig. 4B, lanes 2 and 3). Trace quantities of a protein with an identical mass were observed in culture supernatants containing an excess of glucose (Fig. 4B, lane 4) and, at 2 h prior to the appearance of α-amylase activity, in cultures containing excess starch and limiting glucose (Fig. 4B, lane 1). Production of low levels of the α-amylase monomer is consistent with the low constitutive level of α-amylase activity detected in culture supernatants under these conditions. Synthesis of an additional protein with an apparent mass of 78 kDa was also observed. These results suggest that the effect of glucose on α-amylase production occurs at the level of gene expression.

Repressing and nonrepressing carbon sources. α-Amylase

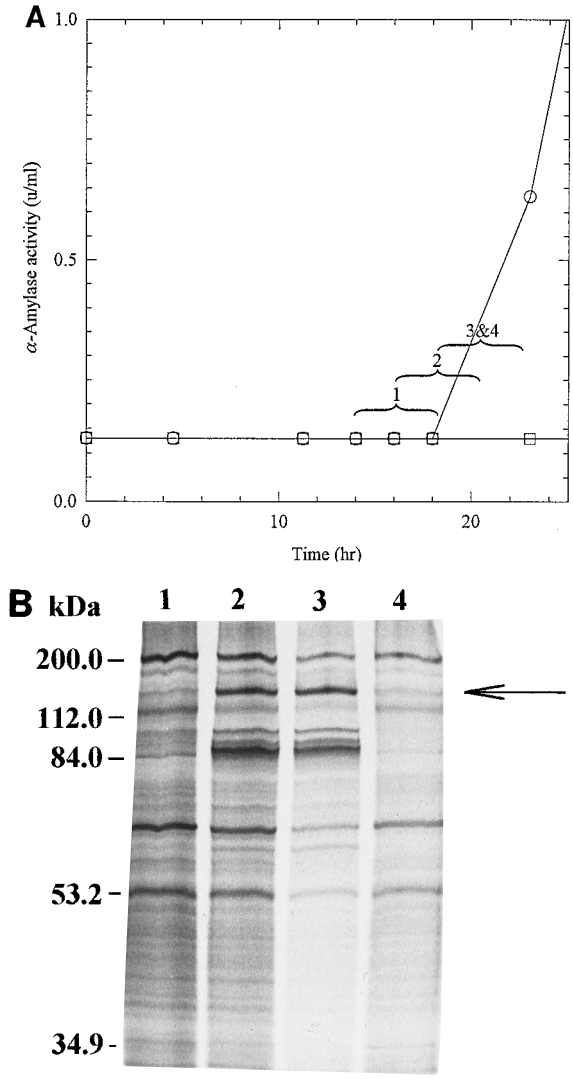

FIG. 4. Synthesis of α-amylase de novo. (A) Time course of culture labeling. Two cultures which utilized either excess starch and limiting glucose (circles) or excess starch and excess glucose (squares) were examined. Levels of secreted α-amylase activity were monitored. Samples of the cultures were labeled periodically with [^{35}S]methionine. The labeling times for the culture containing excess starch and limiting glucose are indicated as 1, 2, and 3; that for the culture containing excess starch and excess glucose is indicated as 4. (B) Autoradiogram of secreted proteins. Portions of the labeled culture supernatants were examined by SDS-PAGE (12.5% gels) and autoradiography. Sample lanes correspond to the labeling times indicated in panel A. The arrow indicates the position of the α-amylase monomer.

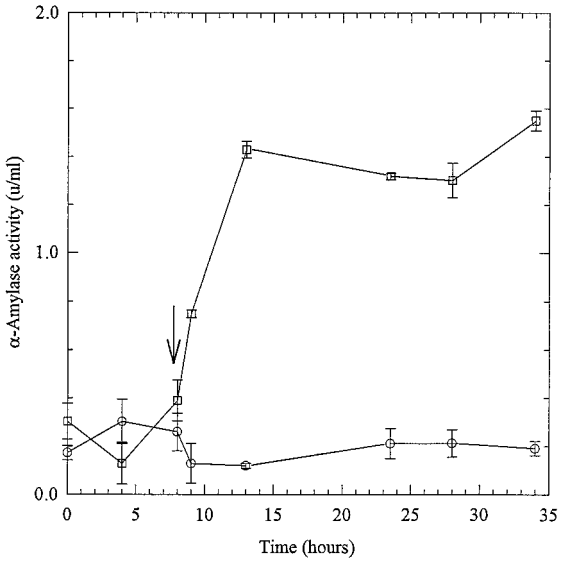

FIG. 5. Induction of α-amylase production during growth on a nonrepressing carbon source. Two cultures containing glutamate as a carbon source were examined for α-amylase production. At the time indicated by the arrow, starch was added to one of the two cultures (squares), while no addition was made to the second culture (circles). The error detected between duplicate samples is shown.

production was detected during growth on glucose even without added starch. Enzyme levels were reduced to 23% of those seen during growth on glucose with added starch. The influence of other carbon sources on α-amylase induction was examined by determining the enzyme levels produced during growth on tryptone as an alternative carbon source with or without added starch. α-Amylase levels remained very low in the culture containing only added tryptone. In the culture containing both tryptone and starch, initial enzyme levels were low. However, at a culture density of 5.6×10^8 cells per ml, enzyme levels increased rapidly to amounts similar to those observed during growth on starch alone. The delayed induction of production of the enzyme could result from the exhaustion of a particular repressing amino acid present in tryptone. To address this possibility, cells were grown on a pool of amino acids consisting of glutamate, aspartate, glycine, serine, and alanine, all present in low amounts (2 mM), as well as excess added starch. A delayed induction in enzyme production again was observed. Of these amino acids, *S. solfataricus* 98/2 utilized only aspartate or glutamate as a sole carbon source. Fully grown batch cultures were achieved with these latter amino acids at final concentrations of 10 mM. Therefore, α-amylase levels were examined during growth on either of these amino acids at a final concentration of 10 mM with added excess starch. Elevated levels of the enzyme were detected only in cultures containing glutamate and starch (1.4 ± 0.003 U/ml), not in cultures containing aspartate with added starch (0.051 ± 0.03 U/ml). Thus, aspartate and glutamate represent repressing and nonrepressing carbon sources, respectively, for starch-mediated enzyme production.

Induction of α-amylase synthesis on a nonrepressing carbon source. The presence of high levels of α-amylase produced during growth on glutamate but not aspartate indicated that glutamate acted as a nonrepressing carbon source for enzyme production. Glutamate was therefore examined for its utility as a carbon source which would permit evaluation of the kinetics of induction of enzyme synthesis by starch addition. This analysis relied on the fact that cell growth and therefore protein synthesis could be sustained by glutamate and was therefore independent of starch availability. To examine this process, two identical cultures were grown with excess glutamate (10 mM) as the sole carbon and energy source. Starch was added to one culture during exponential-phase growth, while no starch was added to the second culture. Enzyme activity was detected in the supernatant of the treated culture immediately following starch addition and reached a maximal level within one cell generation (Fig. 5). In contrast, α-amylase activity in the supernatant of the culture that was not supplemented with starch remained at a low constitutive level of 0.25 ± 0.001 U/ml.

Hierarchal repression of α-amylase production by sole carbon source type. Catabolism of glutamate and aspartate in this

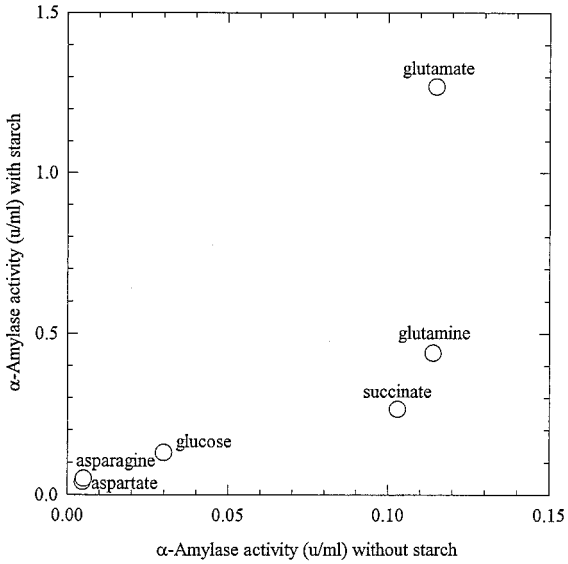

FIG. 6. Hierarchy of carbon sources and α-amylase levels. Cells were grown by using the carbon sources indicated either with or without added starch (0.2% [wt/vol]). Maximal levels of α-amylase activity in the culture supernatants were determined by assaying cultures repeatedly throughout the growth cycle until enzyme levels plateaued.

organism is likely to depend on their conversion into tricarboxylic acid cycle intermediates as well as into other amino acids. The levels of the α-amylase produced during growth on metabolically related sole carbon sources were therefore examined. The effect of the simultaneous addition of starch was also evaluated. Since synthesis of α-amylase is transitory, production of maximal enzyme levels were determined by repeated assay of culture supernatants throughout the growth cycle. A positive correlation was observed between the levels of α-amylase produced during growth on the carbon sources tested, with or without added starch (Fig. 6). Aspartate was the most repressing carbon source, while glutamate was the least repressing. Despite the difference in α-amylase levels observed with these two amino acids, cells appeared to utilize the amino acids with equal efficiency, as generation times were 4.5 h for cells utilizing either carbon source. Asparagine and glutamine, which are metabolically related to aspartate and glutamate, respectively, were also examined. α-Amylase levels were either repressed (asparagine) or derepressed (glutamine), and enzyme levels were again related to the carbon source used for growth with or without added starch. Maximal generation times in assays using these carbon sources were both 7.2 h. Of the tricarboxylic acid cycle intermediates tested, only succinate supported growth as a sole carbon source. No growth was observed on malate, fumarate, α-ketoglutarate, isocitrate, citrate, oxaloacetate, or pyruvate. Glucose and succinate, which had intermediate effects on production of the α-amylase with or without added starch, supported generation times of 7 and 32 h, respectively. Excluded also from examination were the α-amylase substrates dextrin and α-cyclodextrin, as neither carbohydrate supports growth of *S. solfataricus* 98/2. Production of the α-amylase is thus responsive, in a graded fashion, to the sole carbon source used for growth irrespective of cell growth rate. In addition, the maximum range over which α-amylase activity was detected in culture supernatants was 254-fold.

DISCUSSION

The α-amylase reported here is a secreted protein consisting of two identical subunits. It is endo acting and exhibits equal preference for either starch or dextrin. Analysis of culture supernatants indicates this is the major amylolytic activity produced by *S. solfataricus*. Consequently, changes in amylolytic activity detected in culture supernatants are indicative of alterations in this particular enzyme. Levels of the α-amylase in crude culture supernatants varied greatly in response to the sole carbon source used for growth of the organism. Since the secreted enzyme was highly stable, enzyme synthesis but not enzyme turnover is likely to play a major role in mediating the synthesis of extracellular activity. Substrate induction of this enzyme by starch terminated relatively early under all growth conditions tested. Glucose repression of enzyme synthesis was also observed, which indicates that such repression of further enzyme synthesis might be due to the accumulation of starch hydrolytic products, including glucose. The *S. solfataricus* maltase is likely to play an important metabolic role in the accumulation of repressing levels of glucose, as the maltase exhibits considerable activity against dextrins which are the immediate products of α-amylase action (28).

The combination of induction of α-amylase production by starch and repression by glucose suggests that the *S. solfataricus* α-amylase is subject to multiple forms of regulation. Induction of enzyme synthesis by substrate addition is particularly apparent during growth on the nonrepressing carbon source glutamate. Starch addition elicited a large and rapid increase in α-amylase levels, indicating that enzyme production is normally curtailed in the absence of a suitable substrate. Starch induction of α-amylase production also argues for a sensory system for exogenous starch detection. In contrast, most other carbon sources tested precluded the ability of added starch to induce high level α-amylase production. These are thus referred to as repressing carbon sources. The existence of carbon sources which are either repressing or nonrepressing for induction of α-amylase production further extends the similarity between the apparent catabolite repression-like system in *S. solfataricus* and analogous catabolite repression systems found previously in both eukaryotes and prokaryotes.

The regulation of production of α-amylase synthesis seen here appears to be independent of cell growth rate. Both glutamate and aspartate supported maximal rates of growth and resulted in significant differences in enzyme production. In contrast, for the response seen in enteric bacteria, a positive correlation is observed typically between cell growth rate and preferred carbon source type (23). In the related species *Sulfolobus brierleyi*, a relationship has been reported between intracellular levels of glutamate and aspartate and the mode of growth of this organism, whether autotrophic or heterotrophic (15). It is therefore possible that the differential production of α-amylase detected in response to growth on glutamate and aspartate or the related amino acids glutamine and asparagine is pertinent to the role of these amino acids in autotrophic and heterotrophic growth. One mechanism might involve the tricarboxylic acid cycle intermediates α-ketoglutarate and oxaloacetate, produced by the catabolism of these amino acids.

Repression of α-amylase synthesis by glucose represents one type of catabolic control over this enzyme. A second type is the graded production of catabolic enzyme levels resulting from growth on other specific carbon sources. This latter form of control is independent of the presence of starch. These two forms of regulation of α-amylase production may represent a generalized response to carbon source quality or availability, previously observed only in eukaryotes and prokaryotes. Avail-

ability of starch and other plant-derived polysaccharides in the hot springs environment is likely and may transiently provide resident cells with a growth advantage. Regulation of α-amylase synthesis in the hyperthermophile *S. solfataricus* provides an energy-efficient means of utilizing such polysaccharides and indicates that a catabolite repression-like system is present in the archaea.

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