Essential arginine residues in maize starch synthase IIa are involved in both ADP-glucose and primer binding

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Abstract The arginine-specific reagent phenylglyoxal inactivated the activity of maize starch synthase IIa (SSIIa), due to the modification of at least one arginine residue out of a possible 42. The addition of ADPGlc completely protected SSIIa from the inactivation, indicating that arginine may be involved in the interaction of this anionic substrate with SSIIa. However, site-directed mutagenesis of the conserved Arg-214 in SSIIa showed that this amino acid is important for apparent affinity of SSIIa for its primer (amylopectin and glycogen), as evidenced by a marked increase in the K_m for primer upon substitution of this amino acid with no concomitant change in V_{max}, K_m for ADPGlc, or secondary structure. Therefore, Arg-214 of SSIIa appears to play a role in its primer binding.

Key words: Starch; Starch synthase; Chemical modification; Arginine

1. Introduction

Starch, a major storage product in maize endosperm, consists of two major components: amylose and amylopectin. The proportion of the two components and their structures primarily determine the physical-chemical properties of starch. Since industrial demand for specialized starches has been dramatically increasing, efforts are being undertaken to modify the quantity and quality of starch. Understanding the enzymes involved in its biosynthesis, will certainly facilitate this process. To this end, we have undertaken the study of starch synthase (SS) to determine the structure-function relationships involved in its catalysis.

SS is one of three of the major enzyme classes involved in starch synthesis in higher plants, the other two being ADPGlc pyrophosphorylase and branching enzyme [1]. ADPGlc pyrophosphorylase plays a pivotal role in supplying the crucial metabolic precursor, ADPGlc, for starch synthesis, while SS and starch branching enzyme (SBE) primarily determine the starch structure. Multiple forms of SS have been biochemically identified [2–11], while, to date, five cDNA clones have been isolated from maize endosperm (GBSS, SSI, SSIIa, SSIIb, and dull1 [12–15]). Both GBSS and SSI have been purified to homogeneity and these biochemically identified enzymes from maize endosperm have been matched to their respective cDNAs [14,16]. Good evidence exists that the gene product from the dull1 cDNA corresponds to the SSII activity peak biochemically identified by DEAE chromatography [17]. Due to the apparent low abundance of SSIIa and SSIIb, these SS proteins have not been purified directly from maize endosperm. However, these SSIIa and SSIIb have been expressed in *Escherichia coli* and kinetically characterized in detail [18], and in comparing the properties of SSI, SSIIa, and SSIIb, it has been observed that each isoform of SS can be distinguished based on its kinetic properties. For example, maize SSI and maize SSIIb exhibit higher V_{max} values with glycogen as a primer, while SSIIa instead exhibits a higher V_{max} with amylopectin as a primer. Additionally, the specific activity of SSIIb is at least 2–3-fold higher than that for either SSI or SSIIa [16,18].

The arginine-specific reagent phenylglyoxal (PG) has been used as a probe to identify essential arginines involved in the active sites of many enzymes including glutamate dehydrogenase [19], adenylate cyclase [20], hexokinase [21], and alkaline phosphatase [22]. In many cases, an arginine within the active site of an enzyme is many times more reactive than one existing outside of the active site. It is thought that this is due to the reacting residues having lower pK_a values than the usual guanidino group due to the differing microenvironment of the active site [23]. Many of the arginine residues in an active site function to coordinate anionic substrates with their positive charge. Because ADPGlc is one such anionic substrate, we wondered if arginine could play such a role in SS. It has been suggested that Lys-15 in glycogen synthase is the putative binding site for ADPGlc for that enzyme [24]. This lysine residue lies in a conserved motif (KXGG) that is found in all starch synthases [25]; however, this residue has not been directly shown to be involved in ADPGlc binding in SS.

Although maize SSI, SSIIa, and SSIIb exhibit some differences in primer affinity, primer preference, and maximal velocities which may contribute in some way to the different structure(s) of starch during its synthesis, there exists extremely high sequence identity in their catalytic domains [12,15]. This suggests that their catalytic mechanism may be similar. Therefore, to attempt to determine what role arginine may have in the catalysis of starch synthase, we chose SSIIa to be representative of these isoforms. We chose SSIIa because the expression of SSIIa in *E. coli* far exceeds that of SSI or SSIIb [18] and therefore the yield during enzyme purification is greater and larger quantities of material can be obtained.

2. Materials and methods

2.1. Materials

[7-^{14}C]PG was from Amersham. ADP-[U-^{14}C]Glc was synthesized using [U-^{14}C]glucose 1-phosphate (Amersham) and ADPGlc pyrophosphorylase essentially as described [26]. All other supplies and chemicals were the finest grade available from Sigma, or as indicated.

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2.2. Chemical modification of SSIIa with PG

SSIIa in 50 mM HEPES, pH 7.5 was preincubated at 30°C for 3 min, then varying concentrations of PG were added for various times, as indicated in the figure legends. To terminate the modification reaction, 60 mM arginine was added. To separate unreacted [7-^{14}C]PG (4355 μCi/mmol) from SSIIa, a 1 ml Sephadex G-50 spin column was employed as described [27] and protein from the column was determined by the method of Bradford [28].

2.3. Site-directed mutagenesis

Primers used for mutagenesis were:

- ExS98 (5′-CGAGAAGAGGACATCagGTTATGGTTGTG-3′) for the R214Q SSIIa mutant;
- ExS100 (5′-GGTTGTGGTACCAcaGTATGGGGACTATGTG-3′) for R221Q;
- ExS102 (5′-GGAAATCATGAAGCagATGATTTTGTTTTGC-3′) for R284Q;
- ExS104 (5′-GGCTTCATCGGGCaaCTGGATGGACAG-3′) for R492Q;
- ExS106 (5′-CCTCTTTCCGGCACCagCAAGATGACATA-3′) for R269Q;
- ExS117 (5′-GTGATGCCTTCCCagTTCGAGCCCTG-3′) for R567Q;
- ExS144 (5′-CAAGGCTTTAGCGcaAAGAGGACATCGTG-3′) for R210Q;
- ExS146 (5′-GGCTTTAGCGAGAcaAGGACATCGTG-3′) for R211Q;
- ExS148 (5′-CGAGAAGAGGACATaagGTTATGGTTGTGG-3′) for R214K;
- ExS150 (5′-CGAGAAGAGGACATgagGTTATGGTTGTGG-3′) for R214E);
- ExS152 (5′-GCGAGAAGAGGAgctCGTGTTATGGTTG-3′) for H213A;
- ExS183 (5′-GGCTTTAGCGAGAAaAGGACATCGTG-3′) for R211K;
- ExS185 (5′-GGCTTTAGCGAGAgaAGGACATCGTG-3′) for R211E;
- ExS187 (5′-GCGAGAAGAGGAttgCGTGTTATGGTTG-3′) for H213W;
- ExS189 (5′-GCGAGAAGAGGAaAaCGTGTTATGGTTG-3′) for H213K;
- ExS201 (5′-GCGAGAAGAGGAaacCGTGTTATGGTTG-3′) for H213N.

Changed nucleotides are indicated by lower-case letters. Site-directed mutagenesis of the SSIIa cDNA subcloned into the pET21a expression vector (Novagen) (pExS122 [18]) was performed using a PCR in vitro site-directed mutagenesis kit, according to the manufacturer's instructions (Stratagene), using the above primers and their reverse complements. After confirmation of the mutagenesis by DNA sequencing (DNA Sequencing Facility at Iowa State University, Ames, IA), a 300–900 bp fragment, depending on the availability of restriction sites, containing each mutation was re-subcloned into the appropriate sites in pExS122. This was done to ensure that other undesired mutations introduced by PCR were not included in the SSIIa cDNA or vector sequences. The entire subcloned fragments, including the junctions at the restriction sites, were then re-sequenced to ensure no other mutation had occurred during this DNA manipulation.

2.4. Expression, purification, and assay of starch synthase mutants

Expression and purification of wt SSIIa and its mutants was performed as previously described [16,18], with the minor modification that 0.2 M sodium citrate was added to the buffer while loading the amylose affinity column to enhance SS binding to the column. Assay of starch synthase activity, either of crude *E. coli* extracts or of purified preparations of SSIIa, was performed using 5 mg/ml amylopectin and 0.5 M sodium citrate as described [18], and unreacted ADP-[U-^{14}C]Glc was removed with the methanol precipitation method [29]. Assays for kinetic measurements were using a single preparation of enzyme as we had previously demonstrated that several independent preparations of wild type SS had similar specific activities [16,18].

2.5. Circular dichroism (CD) spectra of purified SSIIa mutants

CD spectra were measured by the Iowa State University Protein Facility using a J710 CD spectrometer. The spectra were taken from 190 to 260 nm at room temperature with a protein concentration of 80 μg/ml in 10 mM sodium phosphate, pH 8.0 in a 1 mm cell.

3. Results

3.1. Inactivation of SSIIa by PG

To investigate whether arginine residues are involved in the catalysis of SSIIa, we chemically modified SSIIa with PG and examined its inactivation kinetics. Inactivation of SSIIa activity occurred in a time- and concentration-dependent manner (Fig. 1), with the semi-log plots of inactivation being linear, indicating pseudo-first order kinetics. A double log plot of each rate constant (K) vs. the concentration of PG gives a line with a slope of 1.50 (Fig. 1, inset). This suggests that the inactivation of SSIIa by PG is due to modification of only 1–2 arginines. We then wanted to determine the total incorporation of PG into SSIIa during the modification reaction. By employing [7-^{14}C]PG, we determined the incorporation of PG into SSIIa to be 4 mol [7-^{14}C]PG per mol SSIIa (Fig. 2). Since PG reacts with arginine in either a 1:1 or 2:1 ratio [30], the incorporation data suggest that 2–4 arginines out of a possible 42 arginines in SSIIa are being chemically modified. To identify these modified arginines, hydrolysis of the radioactively labeled [7-^{14}C]PG-SSIIa by protease was followed by high performance liquid chromatography (HPLC) of the resulting peptides. However, it was not successful as the radiolabel came off in the flow through of the chromatography. Perhaps the peptide(s) into which the [7-^{14}C]PG was incorporated was extremely small or the [7-^{14}C]PG-peptide

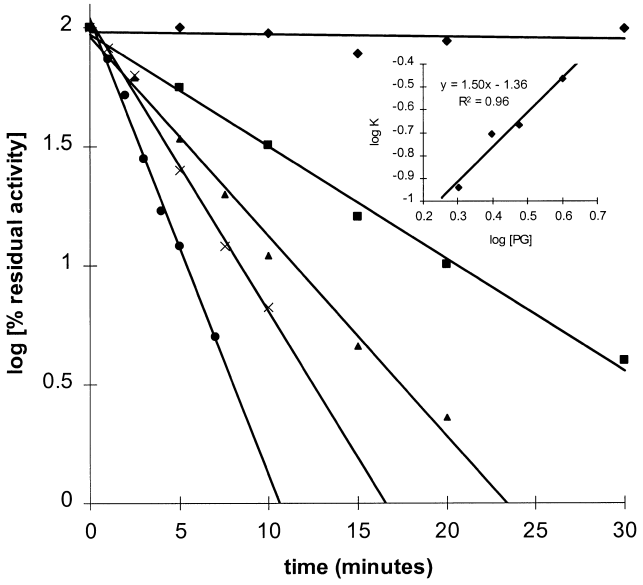

Fig. 1. Time- and concentration-dependent inactivation of SSIIa by PG. 1 μM SSIIa was incubated at 30°C with 2 mM (■); 2.5 mM (▲); 3 mM (×); 4 mM (●); or 0 mM PG (♦) for varying times. The reactions were then terminated with the addition of arginine and the enzyme assayed as described in Section 2. Inset: Double log plot of reaction rate constants. First order rate constants determined from the time inactivation of SSIIa were plotted as a double log plot to give a slope of 1.50.

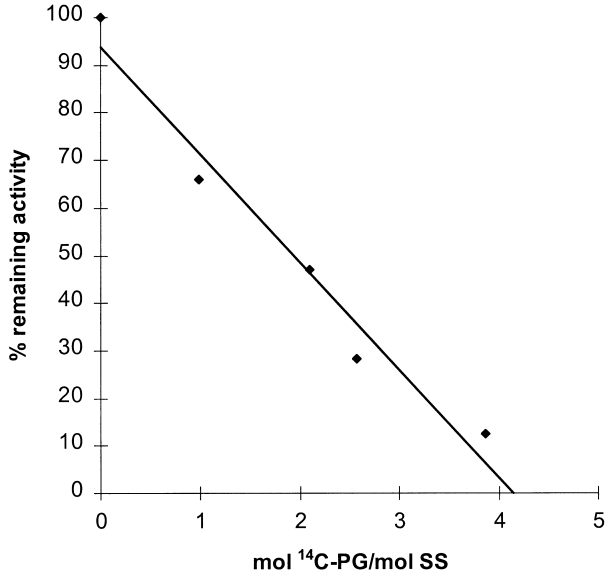

Fig. 2. Incorporation of [7-^{14}C]PG into SSIIa. 4 mM [7-^{14}C]PG was allowed to react with 20 μM SSIIa for various times and the reaction again terminated with the addition of arginine. Unreacted [7-^{14}C]PG was then separated from modified SSIIa, and protein concentration determined as discussed in Section 2. Extrapolation of the trendline indicates that 4 mol [7-^{14}C]PG per mol SSIIa are incorporated during the modification reaction.

linkage was unusually labile under the reverse phase chromatography conditions.

3.2. ADPGlc protection of SSIIa from inactivation by PG

A number of substances including 10 mM ADP, 10 mg/ml glycogen, 5 mg/ml amylopectin, 10 mM ATP, and 50 mM glucose were found not to protect SSIIa from inactivation by PG modification (data not shown). However, ADPGlc was found to protect SSIIa-1 from inactivation by PG (Fig. 3). This protection from inactivation was dependent upon the concentration of ADPGlc, with 10 mM ADPGlc providing SSIIa nearly complete protection from inactivation by PG.

Table 1
Starch synthase activity of SSIIa mutants as measured in crude *E. coli* extract

	Specific activity (nmol/min/mg)	Activity (% of control)
wt	399	100
R210Q	420	105
R211Q	90	22
R211K[a]	164	41
R211E	15	4
H213A[b]	41	10
H213K	36	9
H213W	41	10
H213N[a]	92	23
R214Q[a]	97	24
R214K[a]	300	75
R214E	22	6
R221Q	237	59
R269Q	375	94
R284Q	276	69
R492Q	276	84
R567Q	423	106

[a]Mutant purified for further characterization.
[b]Purification attempted on mutant; however, mutant protein was extremely unstable during purification.

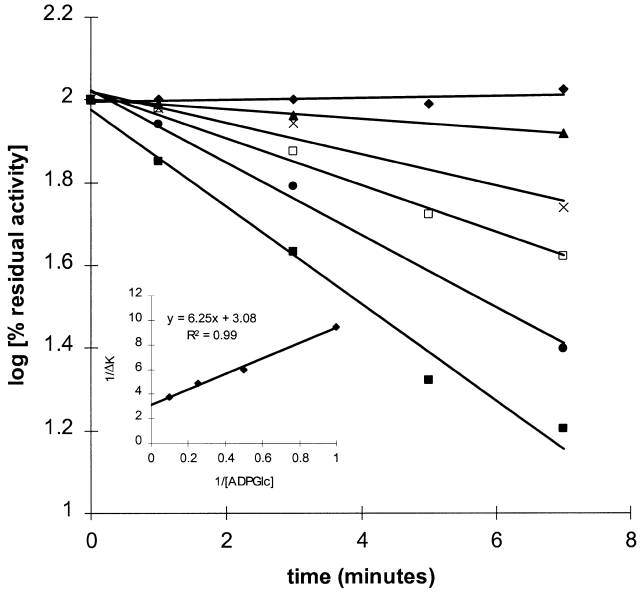

Fig. 3. Effect of ADPGlc on PG modification of SSIIa. 1 μM SSIIa with 4 mM PG was incubated with 10 mM (▲); 4 mM (×); 2 mM (□); 1 mM (●); or 0 mM ADPGlc (◆) for the indicated times. PG reactions were terminated and residual SSIIa activity was determined as before. Inset: Determination of the concentration of ADPGlc required for half maximal protection. The difference in activation rates as compared to control (0 mM ADPGlc) was determined for each concentration of ADPGlc and plotted in a double reciprocal plot. The extrapolated line intercepts the *x*-axis at −0.49, giving the ADPGlc concentration required for half maximal protection at 2.0 mM.

Analysis of a double reciprocal plot of the difference in PG inactivation rates using different concentrations of ADPGlc (Fig. 3, inset) shows that, with 4 mM PG in the inactivation mixture, the concentration of ADPGlc needed for half maximal protection is 2 mM.

3.3. Site-directed mutagenesis of conserved arginines

The chemical modification of SSIIa indicates that at least one arginine residue in the enzyme is important for catalysis and might be near the ADPGlc binding site. Therefore, we undertook the site-directed mutagenesis of conserved arginines in SSIIa in an effort to determine which arginine(s) may fulfill this role. For the initial screen, arginines conserved in maize soluble starch synthases [12,15] were changed to glutamine at positions 210, 211, 214, 221, 269, 284, 492, and 567 in SSIIa. For reference, the lysine that is included in the putative ADPGlc binding sequence (KS/TGGL [24]) lies in position 193 of maize SSIIa (Fig. 4). These site-directed mutants

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SSIIa   193KTGGLGDVVGALPKALARRGHRV
SSIIb   161KTGGLGDVVGALPKALARRGHRV
SSI     107KSGGLGDVCGSLPVALAARGHRV
Waxy     19 KTGGLGDVLGGLPPAMAANGHRV
glg A    15 KTGGLADVIGALPAAQIADGVDA
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Fig. 4. Sequence comparison of residues in close proximity to the putative ADPGlc binding site of SSIIa. Amino acid residues thought to be involved in SS binding to ADPGlc are underlined. Arg-211, His-213, and Arg-214, all mutations which affected SSIIa activity as assayed in the crude *E. coli* lysate, are in boldface. Sequences are taken from [12,15,32,33].

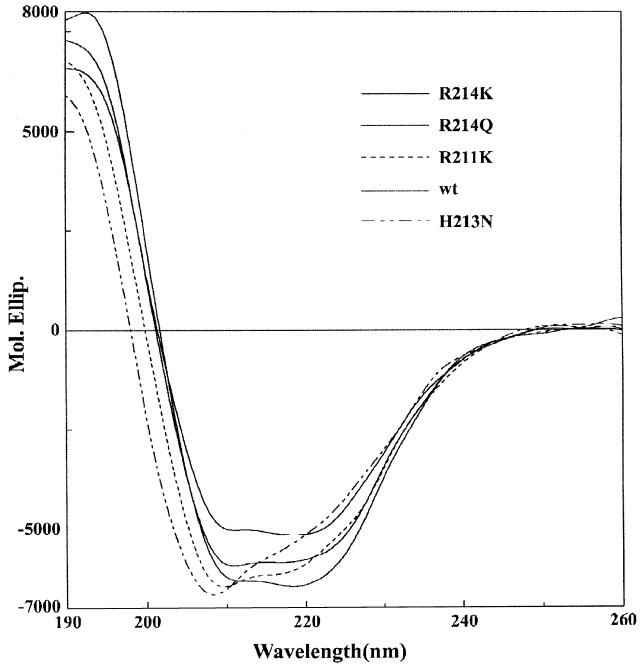

Fig. 5. CD spectra of SSIIa mutants. Mutants are identified by means of the inset legend, with the wt, R214K, and R214Q spectra all being indicated with a solid line as no differences in the shapes of their spectra were noted.

of SSIIa were expressed in the *E. coli* strain BL21(DE3) as described [18], the cells were sonicated, and the cleared lysate was assayed for starch synthase activity. Table 1 shows that the activities of the Arg→Gln mutants at positions 210, 221, 269, 284, 492, and 567 are not significantly lower than that of wt SSIIa, while the mutants R211Q and R214Q exhibited 22% and 24% of the wt enzyme activity. Western blot analysis of the cleared lysates of all expressed mutants using an α-SSIIa antibody [18] showed that expression of the SSIIa mutants did not differ from the level of wt SSIIa (data not shown). Therefore, we decided to concentrate our efforts on the characterization of the function of the arginines at positions 211 and 214. To probe the role of the positive charge of arginine in these mutants, we changed these arginines to lysine (positively charged) and glutamic acid (negatively charged). Changing these arginines back to a positively charged residue resulted in an apparent increase in activity for these mutants (to 41% of wt for R211K and to 75% of wt for R214K) while changing the arginines to a negatively charged residue resulted in essentially dead enzymes (Table 1). Because of the increased activity of the Arg→Lys mutants at these positions, we decided to purify these arginine mutants first.

3.4. Site-directed mutagenesis of His-213

Sequence analysis of maize starch synthases reveals that the sequence ^{211}RGH^{214}R is highly conserved. Many enzymes also contain histidine residues which are catalytically important and His-213 is also highly conserved in maize SSs. Given the proximity of His-213 to two arginines that were possibly important for catalysis (Fig. 4), we proceeded to mutate this histidine as well in an effort to determine the function of the sequence ^{211}RGH^{214}R. As seen in Table 1, the mutant H213A only has 10% of the starch synthase activity as compared to wt when expressed and assayed in *E. coli* extract, indicating that this histidine might indeed be important for catalysis. However, this mutant was extremely unstable and was susceptible to major degradation during the purification procedure. Therefore, we made several other substitutions of His-213 (H213K, H213W, H213N), and chose to purify the H213N mutant since the activity of this mutant in *E. coli* extract was double that of the other mutants at this position (Table 1) and the protein was stable during purification.

3.5. Kinetic characterization of SSIIa mutants

Determination of the kinetic parameters of the wt SSIIa and the purified mutants R211K, R214K, and H213N was carried out using glycogen and amylopectin as primers in the presence of 0.5 M citrate, as previously described [18]. As shown in Table 2, the V_{max} of R211K and H213N were both decreased 2-fold with glycogen or amylopectin as a primer. However, the V_{max} of R214K was not affected when glycogen was used as primer, while its V_{max} was decreased by only 33% when amylopectin was used as primer.

Originally, we employed site-directed mutagenesis on these conserved arginines to identify the residue(s) involved in the binding of ADPGlc because of our previous chemical modification data. If we had affected this particular residue(s) involved in ADPGlc binding, we might presume there would be a change in the K_m for ADPGlc upon site-directed mutagenesis of these residues. Unexpectedly, the K_m for ADPGlc was not affected by any of the mutations in SSIIa (Table 2). Instead, we found significant differences in the primer kinetics for R214K and H213N. R214K exhibited a 6–9-fold increase

Table 2
Kinetic parameters of SSIIa site-directed mutants

		SSIIa-1	R211K	H213N	R214K	R214Q
ADPG kinetics						
With glycogen	V_{max}	19.5 ± 0.1	11.6 ± 0.2	11.8 ± 0.3	20.3 ± 0.3	18.6 ± 0.4
	K_m	0.16 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.20 ± 0.01	0.11 ± 0.01
With amylopectin	V_{max}	30.6 ± 1.2	16.2 ± 0.3	13.9 ± 0.3	21.6 ± 0.3	–
	K_m	0.11 ± 0.01	0.11 ± 0.01	0.17 ± 0.02	0.17 ± 0.01	–
Primer kinetics						
With glycogen	V_{max}	24.0 ± 0.6	9.81 ± 0.10	13.1 ± 0.1	24.3 ± 0.4	18.1 ± 0.4
	K_m	0.14 ± 0.02	0.19 ± 0.04	0.40 ± 0.02	1.28 ± 0.09	1.26 ± 0.12
With amylopectin	V_{max}	29.2 ± 2.3	15.3 ± 0.6	14.8 ± 0.6	22.5 ± 0.6	(23.8 ± 0.8)
	K_m	0.16 ± 0.06	0.21 ± 0.04	0.57 ± 0.09	1.02 ± 0.09	NS

Assays were performed at 37°C as described in Section 2. Data are expressed as the average of three or four independent determinations along with the standard deviation. For ADPGlc kinetics, K_m are expressed in mM ADPGlc. For primer kinetics, K_m are expressed as mg/ml primer, and 3 mM ADPGlc was used in the assays. V_{max} values are in μmol/min/mg protein.

in K_m for primer (glycogen or amylopectin) while a 3-fold increase was observed in the K_m for primer for H213N. The primer kinetics of R211K were not affected by the mutation. Therefore, Arg-214 appears to be important to SSIIa for its primer recognition and/or binding. This is further evidenced by the kinetic characterization of purified R214Q, in which the positively charged arginine is replaced by the neutral glutamine. The R214Q mutant again exhibited a 7-fold increase in K_m for glycogen with no change in K_m for ADPGlc (Table 2). However, no K_m could be determined for the R214Q mutant for amylopectin as apparent affinity for this primer had been affected so greatly that saturating concentrations of amylopectin could not be reached.

3.6. CD spectra of purified SSIIa mutants

It could be argued that the changes in kinetics observed for the SSIIa mutants are not due to the change in an amino acid important for catalytic function but could be due to drastic conformational changes in the protein upon the introduction of a different amino acid. To determine if this was the case, CD spectra on the purified SSIIa mutants were compared to that of wt. For the R214K and R214Q mutants, the CD spectra were the same as wt (Fig. 5). However, a slight shift in the CD spectrum was observed for the R211K mutant, while a more significant shift in the H213N CD spectrum was found.

4. Discussion

There have been numerous examples of enzymes utilizing the positively charged guanidino group of arginine to orient anionic substrates correctly in the active site of the enzyme [31]. One approach to probe the role of arginine in these proteins is chemical modification of arginine with PG while another approach is site-directed mutagenesis of those arginines. We have employed both strategies to show that arginine most likely is involved both in ADPGlc binding and in primer affinity and/or binding to SSIIa. Initially, we used PG to preferentially modify arginine residues in SSIIa and found that inactivation of SSIIa occurred most likely due to the modification of only 1–2 arginines. ADPGlc was the only substance tested to be found to completely protect SSIIa from inactivation by PG. These data suggest that 1–2 arginines are involved in the binding or interaction with ADPGlc during catalysis. Identification of these modified arginines was not successful, preventing a conclusion as to which specific arginine(s) are important in the binding of ADPGlc.

We then employed site-directed mutagenesis of conserved arginines in SSIIa in an effort to determine which arginine(s) may be important in SS catalysis. Changes at two positions, Arg-211 and Arg-214, decreased the activity of SSIIa as assayed in the expressed *E. coli* extract. Given its proximity to these two arginines, the function of His-213 in SSIIa was also examined. After purification and kinetic characterization of these mutants, we found that R211K exhibited no change in K_m for primer, while R214K and H213N showed significant increases in their K_m values for primer as compared to wt. Unexpectedly, none of the purified mutants exhibited a change in their apparent affinity for ADPGlc. Therefore, it does not seem likely that the 1–2 arginines being modified by phenylglyoxal in SSIIa are Arg-211 or Arg-214. The arginine(s) responsible for the inactivation of SSIIa upon its modification with PG remain as yet unidentified; perhaps these arginines are as not as conserved in all SSs as originally thought and the arginine important in ADPGlc binding. was passed over in this study. Alternatively, the arginine important in ADPGlc binding might have been one changed in this study but the mutant did not show decreased activity as assayed in the crude lysate.

Instead, we propose that Arg-214 is somehow important to SSIIa for its primer recognition and/or binding. Since the substitution of Arg-214 with Lys in SSIIa resulted in such an increase in the apparent affinity of the enzyme for primer, we purified the R214Q mutant of SSIIa to determine if substitution of a neutral amino acid at this position would have any additional effect on the kinetics of the enzyme. With amylopectin as a primer, the apparent affinity of R214Q for this primer was so affected that saturating concentrations of amylopectin could not be obtained and the K_m could not be determined. Therefore, it does appear that the positive charge at Arg-214 is important for recognition/binding of amylopectin, the preferred substrate of SSIIa [18].

To determine if the changes in kinetics observed for the site-directed mutants of SSIIa were due to a change in an amino acid important for its function or due to drastic changes in protein structure upon introduction of the mutation, the CD spectra of the mutants were compared to that of wt. While the CD spectra of the R214K and R214Q mutants were the same as observed for wt enzyme, a slight shift in the CD curve was observed for the R211K mutant and a more pronounced difference in the CD spectrum was observed for the H213N when compared to wt. Because of the CD spectrum of H213N, we cannot unequivocally come to the conclusion that H213N is important for primer binding or catalysis. The fact that pronounced conformational changes are introduced into SSIIa upon substitution of His213 may be an indication that this amino acid is somehow important for its structural stability. This may be a clue as to why the H213A mutant was extremely susceptible to degradation during its purification.

In summary, although it cannot be concluded which arginine(s) are important in the interaction between the anionic substrate ADPGlc and SSIIa, we have strong evidence that 1–2 arginines in SSIIa function in this capacity based on its chemical modification with the arginine-specific reagent phenylglyoxal. We have, however, definitely identified by site-directed mutagenesis Arg-214 as a critical amino acid for the recognition and/or binding of primer to SSIIa. We are confident that the decrease in apparent affinity of this enzyme for primer upon substitution of Arg-214 is not due to conformational changes induced by the mutation for two reasons. First, the CD spectrum of two mutants in this position, R214K and R214Q, are not significantly different from that of wt SSIIa. Second, the only kinetic parameter which Arg-214 mutants differ from that of wt is their K_m values for primer. The V_{max} of R214K and R214Q, as well as their K_m values for ADPGlc, remain unchanged. It appears that the guanidino group of Arg-214, not just the positive charge of arginine, is important for the interaction of SSIIa and primer, since substitution of Arg-214 with Lys still results in a marked decrease in apparent primer affinity. It remains to be seen if substitution of other amino acids at this position could result in an active enzyme which has an altered, or better, affinity for a particular primer. Of course, the native primer for SS is not

pure glycogen or amylopectin; therefore, more investigation concerning the exact interactions between SS and native primers is needed, and this is currently in progress.

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