Dynamic Domains of DNA Polymerase α in Regenerating Rat Liver†

Harold C. Smith and Ronald Berezney*

ABSTRACT: Intranuclear redistributions of DNA polymerase α activity (the presumptive replicative enzyme) are observed preceding and during in vivo replication in regenerating rat liver. The most marked induction of in vitro enzyme activity occurs coincident with the onset of in vivo replication and is seen in the high salt resistant domain of the nuclear matrix. Moreover, DNA polymerase α endogenous and exogenous template activities begin to redistribute prereplicatively from bulk chromatin directly to the high salt resistant matrix. These prereplicative subnuclear events are detectable prior to the induction of total cellular DNA polymerase α and appear to continue throughout replication. Maximal percent recovery of the total nuclear activity on the high salt resistant matrix (~30%) occurs coincident with and during the time when in vivo replication rates are maximum. At the conclusion of replication, DNA polymerase α activities shift back into the bulk chromatin and are no longer high salt resistant. In contrast, DNA polymerase β (presumptive repair enzyme) endogenous and exogenous template activities are randomly distributed in the nuclear subfractions throughout the proliferative response. We conclude that DNA polymerase α is dynamically assembled or activated on the nuclear matrix and that this process is initiated before the onset of in vivo replication.

Recent studies have brought to light the residual nuclear matrix as central in the higher organization of DNA in the nucleus (Georgiev et al., 1978; Dijkwel et al., 1979; Vogelstein et al., 1980; Basler et al., 1981; Berezney, 1981). Of major importance is the association of DNA with the matrix in the form of anchored supercoiled loops (Georgiev et al., 1978; Vogelstein et al., 1980; Berezney & Buchholtz, 1981a). This association of DNA loops is maintained throughout the cell cycle where components of the nuclear matrix are conserved in mitotic chromosomes as the chromosome scaffold (Adolph et al., 1977; Paulson & Laemmli, 1977). Though it is apparent that the association of DNA with the matrix is of structural significance, the precise functional relationships between the matrix and other nuclear regions or domains are generally not known. Several studies, however, have shown that DNA closest to the matrix attachment sites is enriched in newly replicated DNA (Berezney & Coffey, 1975, 1976; Dijkwel et al., 1979; Pardoll et al., 1980) and topological models of replication have been proposed wherein the DNA loop domains move through matrix-bound replication complexes or "replisomes" (Dijkwel et al., 1979; Pardoll et al., 1980; Berezney & Buchholtz, 1981b). In support of these models we previously demonstrated substantial amounts of DNA polymerase α (the presumptive replicative enzyme) but not DNA polymerase β (the presumptive repair enzyme) tightly bound to the nuclear matrix at the time of in vivo replication and have characterized the nuclear matrix as an in vitro system for studying DNA synthesis (Smith & Berezney, 1980, 1982). What remains unclear is exactly when DNA polymerase α becomes high salt resistant (matrix bound) and the consequences of this binding for the other intranuclear chromatin domains and the replicative apparatus.

In this paper we study the distribution of both DNA polymerase α and β endogenous and exogenous template activities in various nuclear subfractions or domains during the proliferative response of liver regeneration. Our results demonstrate intranuclear shifts of DNA polymerase α activities during in vivo replication. In particular, we observe marked redistributions of enzyme activity from chromatin domains to the high salt resistant nuclear matrix which are initiated prior to the onset of in vivo replication and the induction of total cellular DNA polymerase α activity. DNA polymerase β activity did not show these intranuclear redistributions. These findings provide evidence for the prereplicative assembly and/or activation of DNA polymerase α on the nuclear matrix and represent the first detailed analysis on the dynamic nature of the nuclear domains for this enzyme activity.

Materials and Methods

Nuclear Isolation and Fractionation. Liver nuclei were prepared from either normal or two-thirds hepatectomized male rats (Sprague-Dawley, 200–250 g; King Animal Labs) as previously described (Smith & Berezney, 1980, 1982). Details of the procedure for nuclear matrix isolation have been previously published (Smith & Berezney, 1982). In brief endogenously digested nuclei are separated by low ionic strength extractions [0.2 mM $MgCl_2$ and 10 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.4] into a soluble bulk chromatin fraction (75–80% of total nuclear DNA) and a remaining low salt nuclear matrix (20–25% of total nuclear DNA). The low salt matrix structures are then extracted with high ionic strength (2 M NaCl, 0.2 mM $MgCl_2$, and 10 mM Tris, pH 7.4) to release a high salt soluble chromatin fraction (18–23% of total nuclear DNA). The high salt resistant nuclear matrix contains 1–2% of the total nuclear DNA and is subsequently treated with 0.4% Triton X-100 (0.2 mM $MgCl_2$ and 10 mM Tris, pH 7.4) to remove nculear envelope components. The final nuclear matrix corresponds to nuclear matrix III structures according to the terminology of Berezney (1979).

In Vivo DNA Replication. In vivo incorporation of [*methyl*-^{3}H]thymidine (50–80 Ci/mM; New England Nuclear) into DNA and analysis of the labeled DNA were performed as previously described (Berezney & Buchholtz, 1981b). All measurements were made on livers pooled from three animals for each time point.

In Vitro DNA Synthesis. DNA polymerase α and β endogenous and exogenous template activities were assayed as previously reported (Smith & Berezney, 1982).

† From the Division of Cell and Molecular Biology, Department of Biological Sciences, State University of New York, Buffalo, New York 14260. *Received February 25, 1983.* This work was supported by U.S. Public Health Service Grant GM-23922.

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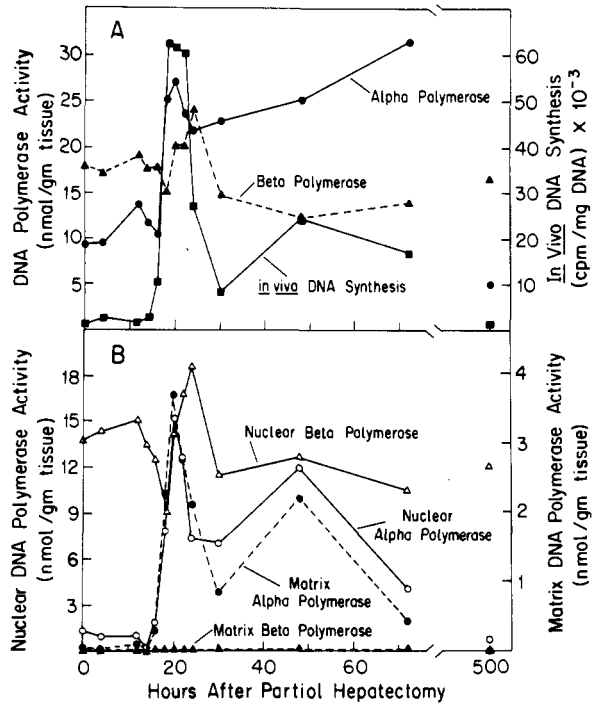

FIGURE 1: Induction of total cellular, nuclear, and matrix-bound DNA polymerase α and β activity during liver regeneration. (A) Total cellular in vivo DNA synthesis and DNA polymerase α and β exogenous template activities measured at different intervals following partial hepatectomy. Total cellular DNA polymerase α and β exogenous template activities are the arithmetic sum of total nuclear and cytoplasmic activity. The cytoplasmic fraction is the postnuclear supernatant obtained after the initial low-speed centrifugation of the liver homogenate [see Basler et al., (1981) and Berezney & Buchholtz (1981b) for details]. Total nuclear activities were corrected for nuclear recovery (generally, 60–80% of total nuclei) based on DNA recovery measurements. (B) Total nuclear and high salt resistant matrix DNA polymerase α and β exogenous template activities. Values represent the mean of three to six determinations from two separate experiments. The ranges for the standard error of the means were within 1.0–8.0% of the mean and 1.0–10.0% of the mean for values present in (A) and (B), respectively.

Results

Induction and Redistribution of Total Cellular, Nuclear, and Matrix-Bound DNA Polymerase α. Total cellular DNA polymerase α exogenous template activity in regenerating liver increased in parallel with the onset of in vivo replication (Figure 1A). The high salt resistant, matrix-bound DNA polymerase α exogenous template activity showed the highest level of induction coincident with the peak of in vivo DNA replication (Figure 1B). Increases of total nuclear DNA polymerase α exogenous template activity also coincided with in vivo replication but were not as pronounced as that of the matrix-bound enzyme (Figure 1B).

The levels of nuclear and matrix DNA polymerase α exogenous template activities (Figure 1B) followed the same cyclic pattern as in vivo replication which dropped to very low levels at 24–30-h posthepatectomy (Figure 1A). In contrast, total cellular DNA polymerase α activity remained elevated after the first peak and did not show a cyclic pattern. Total cellular, nuclear, and matrix-bound DNA polymerase α activity returned to levels similar to those in normal liver within 3 weeks of regeneration.

In addition to the induction of DNA polymerase α exogenous template activity, we measured an increase in the percent of total cellular exogenous template activity in nuclei from $\leq$14% in normal nuclei to a maximum of 56% during the period of maximum in vivo replication (Figure 2). Subsequently, the percent of total activity in nuclei decreased and reached values comparable to those of normal liver by 72 h. Coincident with both in vivo replication and the increased percent of total cellular DNA polymerase α activity in the nucleus was a parallel increase in the percent of total nuclear DNA polymerase α activity in the high salt resistant matrix from <5% to a maximum of 30% at 18–24-h posthepatectomy (Figure 2). Similar increases were also observed in the DNA polymerase α endogenous template activity bound to the matrix (data not shown). Increases of total nuclear enzyme bound to the matrix were evident as early as 12 h. Since the first wave of in vivo DNA replication was initiated 14–16-h posthepatectomy, the onset of this nuclear redistribution was prereplicative (compare Figures 1 and 2).

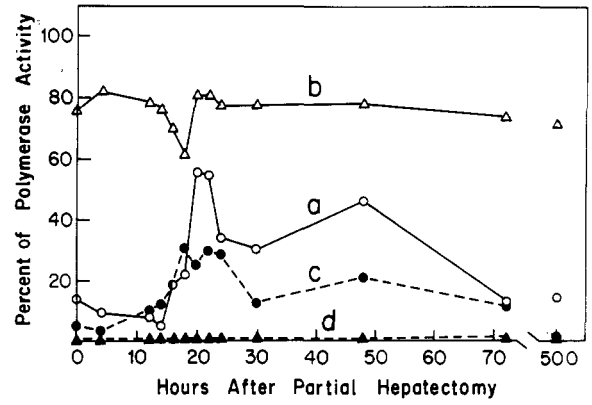

FIGURE 2: Percent recovery of DNA polymerase α and β activities in total nuclear and high salt resistant matrix domains. The percents of total cellular DNA polymerase α and β exogenous template activities recovered in isolated nuclei were calculated at different intervals following partial hepatectomy. Similarly, the percent of total nuclear polymerase activity recovered in the high salt resistant nuclear matrix is also plotted. All values were corrected for nuclear recovery. Values represent the mean of three to six determinations from two separate experiments. The range for the standard error of the means was within 1.0–9.0% of the mean for all values shown. (a) Percent of total cellular DNA polymerase α activity recovered in isolated nuclei (○); (b) percent of total cellular DNA polymerase β activity recovered in isolated nuclei (△); (c) percent of total nuclear DNA polymerase α activity recovered in high salt resistant nuclear matrix (●); (d) percent of total nuclear DNA polymerase β activity recovered in high salt resistant nuclear matrix (▲).

In contrast to these findings, total cellular DNA polymerase β exogenous template activity remained generally unaltered from normal liver values throughout the regenerative response. There was, however, a slight 1.4-fold increase over normal liver values measured at 24-h posthepatectomy. The majority of the total cellular DNA polymerase β exogenous template activity remained within isolated nuclei throughout liver regeneration (Figure 2). Despite this nuclear localization, and unlike DNA polymerase α, DNA polymerase β was depleted in isolated high salt resistant nuclear matrices (Figure 1B). Less than 1% of the total nuclear exogenous template activity was recovered with this matrix fraction.

Prereplicative Shift of DNA Polymerase α from Bulk Chromatin to Low Salt Matrix Domains. The preceding results suggest that dynamic transitions occur in the binding and/or intranuclear localization of DNA polymerase α which are closely coordinated with in vivo replication. To further study the temporal occurrence of this phenomenon and its relationship to other nuclear domains, we measured DNA polymerase α activities during liver regeneration in the various chromatin and matrix fractions. All chromatin fractions showed elevations and depressions in enzyme activity in coordination with cycles of in vivo replication. Closer analysis revealed that the levels of enzyme induction were nonrandom

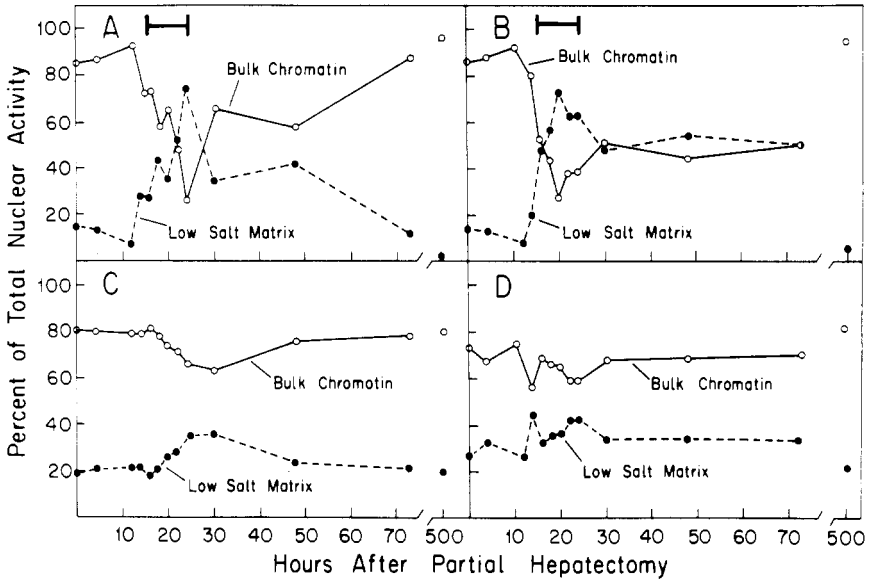

FIGURE 3: Percent distribution of DNA polymerase α and β exogenous and endogenous template activities between bulk chromatin and low salt matrix domains. DNA polymerase α and β activities were measured in nuclei fractionated into bulk chromatin (75–80% of total nuclear DNA) and low salt matrix structures (20–25% of total DNA) at different intervals following partial hepatectomy. The distribution of total polymerase activity into each fraction was then calculated on a percentage basis. Values represent the mean of three to six determinations from two separate experiments. The ranges for the standard error of the means were within 1.0–10.0% and 1.0–9.0% of the values shown in (A), (B) and (C), (D), respectively. (A) DNA polymerase α exogenous template activity, (B) DNA polymerase α endogenous template activity, (C) DNA polymerase β exogenous template activity, and (D) DNA polymerase β endogenous template activity. The brackets in panels A and B encompass the duration of the first wave of in vivo replication (16–24-h posthepatectomy).

with respect to chromatin and matrix fractions. The low salt matrix showed a more pronounced induction of DNA polymerase α activity than bulk chromatin. DNA polymerase exogenous and endogenous template activities in bulk chromatin were elevated 2–3-fold over normal liver values during replication, whereas the low salt matrix showed a striking 9- and 23-fold elevation in the corresponding polymerase activities. The direct consequence of the differential rates of induction was a shift in the intranuclear distribution of DNA polymerase α activity from bulk chromatin to the low salt matrix (Figure 3). Importantly, this massive shift of activity began prereplicatively (12–14 h). As the relative percent of total nuclear activity in the low salt matrix increased, the percent in bulk chromatin dropped precipitously. This reciprocal relationship persisted until 24 h, when the first wave of in vivo replication was concluding. After this time, DNA polymerase α activity began to redistribute such that the majority of the nuclear activity again resided within the bulk chromatin by 30 h. The distribution of DNA polymerase α activity in bulk chromatin and low salt matrix returned to normal liver values within 3 weeks of regeneration.

In contrast, DNA polymerase β exogenous and endogenous template activities did not show marked redistributions within bulk chromatin and low salt matrix fractions (Figure 3C,D). The majority of nuclear DNA polymerase β activities were recovered with the bulk chromatin fraction throughout regeneration.

Prereplicative Shift of DNA Polymerase α to the High Salt Resistant Matrix Domain. The low salt matrix is composed of at least two domains which can be biochemically fractionated into a high salt soluble chromatin and a high salt resistant final matrix. Analysis of the distribution of DNA polymerase α within these two subfractions showed that the prereplicative redistribution of enzyme activity from bulk chromatin to the low salt matrix actually involved a direct shift to the high salt resistant matrix (compare Figures 3 and 4). The relative proportion of enzyme activity in the high salt soluble chromatin fraction decreased to a minimum during this prereplicative phase (Figure 4); 100% of the total low salt matrix exogenous template activity was high salt resistant for 12–16-h posthepatectomy. This was followed by a gradual decrease from 70 to 40% over the next 8 h (16–24-h posthepatectomy). The high salt resistant, endogenous template activity decreased from 100% at 12 h posthepatectomy to a steady-state value of 30% during the 16–24-h period of in vivo replication. At the conclusion of replication (30 h), both exogenous and endogenous template activities became increasingly high salt extractable. This postreplicative shift to a high salt sensitive state was accompanied by further redistributions of enzyme activities. These included a shift from low salt matrix to bulk chromatin (Figure 3A,B) and a decrease in the relative amount of total cellular enzyme in the nucleus (Figure 2). The relative proportion of DNA polymerase α activities in the high salt soluble fraction returned to normal liver levels within 3 weeks of regeneration.

Low salt matrix DNA polymerase β exogenous and endogenous template activities were almost entirely restricted to the high salt soluble fraction throughout the regenerative process (Figure 4C,D). No redistributions of DNA polymerase β exogenous or endogenous template activities were detected.

Discussion

Previous investigators have proposed that changes in the amount of nuclear DNA polymerase activity throughout the cell cycle are directly correlated with changes in the amount of tightly bound nuclear enzyme (Littlefield et al., 1963; Gold & Helleiner, 1964; Loeb et al., 1969; Wallace at al., 1971; Lynch & Lieberman, 1973; Chiu & Baril, 1975; Seki & Mueller, 1976; Ono et al., 1979). Nuclear and chromatin in vitro studies have provided support for this proposal by demonstrating that the nuclear enzyme activity most resistant to salt extraction shows the strongest correlation with DNA replication (Lindsay et al., 1970; Wallace et al., 1971; Lynch & Lieberman, 1973; Seki & Mueller, 1976; Nishioka et al., 1977; Ono et al., 1978, 1979; Matsukage et al., 1979; Tanuma et al., 1980). The data in this study are consistent with these

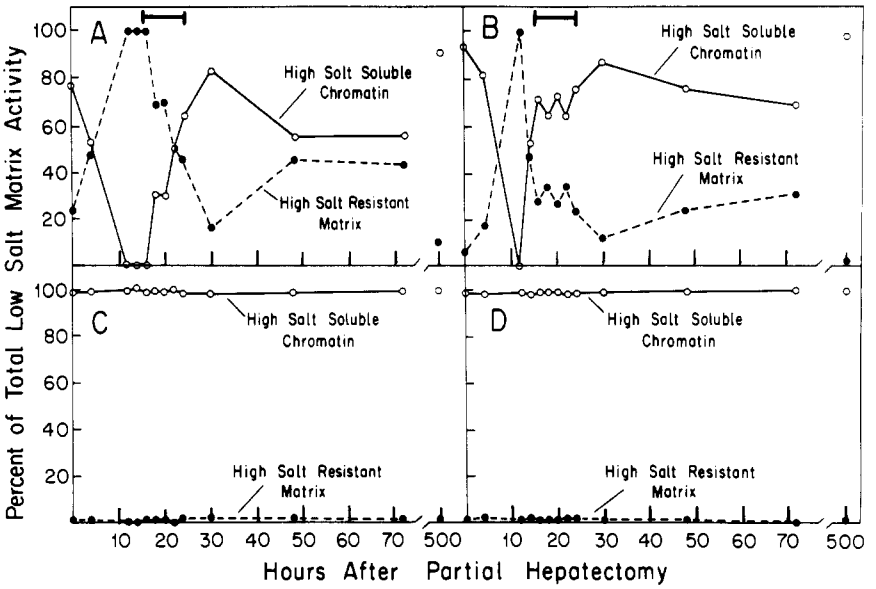

FIGURE 4: Percent distribution of DNA polymerase α and β activities were measured in the low salt matrix fractionated into high salt soluble chromatin (18–23% of total nuclear DNA) and the high salt resistant matrix (1–2% of total DNA) at different intervals following partial hepatectomy. The distribution of total polymerase activity into each fraction was then calculated on a percentage basis. Enzyme activity in the high salt soluble chromatin was estimated by the total activity in the low salt matrix minus the activity of the high salt resistant matrix. Values represent the mean of three to six determinations from two separate experiments. The ranges for the standard error of the means were within 1.0–10.0% and 1.0–5.0% of the values shown in (A), (B) and (C), (D), respectively. (A) DNA polymerase α exogenous template activity, (B) DNA polymerase α endogenous template activity, (C) DNA polymerase β exogenous template activity, and (D) DNA polymerase β endogenous template activity. The brackets in panels A and B encompass the duration of the first wave of in vivo replication (16–24-h posthepatectomy).

previous studies and identify the salt-resistant, tight-binding nuclear domain as the nuclear matrix.

Several laboratories have demonstrated that the nuclear matrix is the site of in vivo DNA replication in eucaryotic cells (Berezney & Coffey, 1975, 1976; Dijkwel et al., 1979; Pardoll et al., 1980; Berezney & Buchholtz, 1981b; Hunt & Vogelstein, 1981) and have proposed models in which they predict that the replicative enzyme DNA polymerase α and perhaps entire replicational complexes or replisomes are associated with the matrix during DNA replication (Dijkwel et al., 1979; Pardoll et al., 1980; McCready et al., 1980; Berezney & Buchholtz, 1981b). In this regard, our laboratory previously reported significant levels of DNA polymerase α associated with rat liver nuclear matrix, and it was proposed that functional replisomes are dynamically assembled on the matrix during in vivo replication (Smith & Berezney, 1980, 1982). This study supports these hypotheses, although the data cannot distinguish between actual assembly of enzyme on the matrix or activation of "prebound enzyme". Moreover, the prereplicative shift of DNA polymerase α activity to the matrix further suggests a possible role of this salt-resistant domain of the matrix in the regulation of DNA replication. Importantly, DNA polymerase β (a presumptive repair enzyme) does not demonstrate these intranuclear redistributions. The majority of nuclear DNA polymerase β activity remains in the bulk chromatin throughout the regenerative response with only trace amounts recovered in the high salt resistant nuclear matrix.

T antigen and DNA polymerase α association with the nuclear matrix have also been demonstrated in polyoma (Buckler-White et al., 1980; A. J. Buckler-White et al., personal communication) and SV-40 (Jones & Su, 1982) viral-infected cells. Although it is not known whether other replicative factors are assembled on the nuclear matrix, Reddy & Pardee (1980) demonstrated in Chinese hamster fibroblast cells that in addition to a DNA polymerase activity, several enzymes involved in DNA precursor metabolism redistribute prereplicatively from the cytoplasm to the nucleus and are associated in a rapidly sedimenting nuclear complex.

In conclusion, this study has shown prereplicative shifts in DNA polymerase α activity among nuclear subfractions. Most striking is the shift of polymerase α activity to the nuclear matrix which may represent a direct conversion of high salt soluble enzyme activity to a high salt resistant, matrix-bound state. In future studies we hope to utilize a recently developed nuclear matrix in vitro DNA synthesis system (Smith & Berezney, 1982) to probe the mechanism(s) behind these prereplicative events and to investigate the possible role of the nuclear matrix in coordinating the structural and functional properties of eucaryotic DNA replication.

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Registry No. DNA polymerase, 9012-90-2.

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Articles

On the Mode of the Alkali Light Chain Association to the Heavy Chain of Myosin Subfragment 1. Evidence for the Involvement of the Carboxyl-Terminal Region of the Heavy Chain†

Morris Burke,* Mathoor Sivaramakrishnan,‡ and Vedhachalam Kamalakannan

ABSTRACT: Evidence is presented that the removal of the alkali light chain subunit from myosin subfragment 1 results in the exposure of a site (or sites) at the carboxyl-terminal region of the heavy chain that is rapidly digested by both trypsin and α-chymotrypsin. In the case of trypsin digestion, cleavage at this site proceeds at a much higher rate than cleavages at the two other sensitive regions located in the interior of the primary structure of this chain. This initial cleavage is responsible for the generation, on further digestion with trypsin, of a carboxyl-terminal fragment about 3000 daltons smaller than the corresponding fragment formed by digestion of subfragment 1. The ability of the heavy chain to reassociate with alkali light chain at 4 °C in the presence of MgATP is essentially abolished by cleavage at this exposed site by either trypsin or chymotrypsin. These observations indicate that the alkali light chain is binding to, or is capable of perturbing, a region of the heavy chain adjacent to the subfragment 1/subfragment 2 "hinge" region and support recent proposals that both the DTNB light chain and the alkali light chain may be interacting and may be modulating this flexible region of the cross bridge.

Previous studies have shown that under certain solvent conditions the stability of the subunit interactions in myosin and myosin subfragment 1 (SF1)[1] is sufficiently labile to heat that dissociation can be detected (Higuchi et al., 1978; Wikman-Coffelt et al., 1979). However, in the presence of the MgATP substrate no subunit dissociation appears to occur at

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‡ Present address: Department of Neurology, Baylor College of Medicine, Houston, TX 77030.

[1] Abbreviations: TPCK, L-1-(tosylamido)-2-phenylethyl chloromethyl ketone; SF1 and SF2, myosin subfragments 1 and 2, respectively; SF1(A1) and SF1(A2), myosin SF1 containing A1 and A2 light chains, respectively; T1 and T2, trypsin digestion sites on the SF1 heavy chain, with T1′ near the carboxyl-terminal end; TF1, TF2, and TF3, tryptic fragments of SF1 heavy chain numbered in sequential order from the carboxyl-terminal end of the subfragment 1 heavy chain; STI, soybean trypsin inhibitor; NEM, *N*-ethylmaleimide; Tris, tris(hydroxymethyl)-aminomethane; $NaDodSO_4$, sodium dodecyl sulfate; ATPase, adenosinetriphosphatase.

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