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Changes in the Activity of Nuclear Protein Kinase CK2 During Rat Liver Regeneration

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Protein kinase CK2 has been found in two nuclear fractions obtained after treatment of purified rat liver nuclei with nucleases (S1 fraction) and subsequently with 1.6 M NaCl (S2 fraction). In both fractions three isoforms of the α subunit were identified. Two of them corresponded to the classical α and α' subunits, whereas the identity of the third one (α_3) remains unknown. In the S1 fraction two peaks of CK2 activity were detected at 6 h (5.5 fold) and 24 h (1.9 fold) after partial hepatectomy, whereas no significant changes were found in the S2 fraction. At 6 h after laparatomy a much lower increase of CK2 in S1 fraction was also detected (2.5 fold). The increases in CK2 activity found at 6 h after hepatectomy or laparatomy were accompanied with rises in the amount of the α_3 subunit.

Protein kinase CK2 (also known as casein kinase 2) is a serine/threonine kinase composed by two catalytic subunits: α and α' of 42-44 kDa and 38 kDa, respectively and a regulatory β subunit of 28 kDa (1). In vivo CK2 exists as a tetrameric holoenzyme composed of either $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$ or $\alpha'_2\beta_2$ (2,3). CK2 can use both ATP and GTP as phosphate donnors, what distinguishes it from other protein kinases (4). CK2 has been highly conserved throughout evolution and is broadly distributed in eukaryotic cells, although its function and regulation is not yet fully understood. CK2 is located in the cytosol and nucleus and some of its substrates are enzymes involved in nucleic acid synthesis, transcription factors, signal transduction proteins and protein synthesis factors (1). Previous works have demonstrated that CK2 is involved in cell growth and proliferation (4-6) and that the mitogenic signal induces the translocation of CK2 from cytosol to the nucleus (6,7). We have investigated here the changes in the amount and activity of CK2 in several nuclear subfractions from rat liver cells during liver regeneration induced by a partial hepatectomy (PH). Nuclear CK2 can be extracted from rat liver cells nuclei by DNase or/and RNase digestion and subsequently with 1.6 M NaCl. The results revealed two peaks of CK2 activity at 6 h and 24 h post-PH in the nuclease-extracted fraction whereas no significant changes were observed in the salt-extracted fraction. The peak found at 6 h is much higher than that observed at 24 h. Interestingly, the increased CK2 activity at 6 h correlates very well with the elevation of the amount of a low molecular weight isoform of the α subunit in both regenerating and sham-operated rats.

MATERIALS AND METHODS

Animals. Male 200-250 g Sprague-Dawley rats were used for the experiments. Partial hepatectomy (PH) was performed as described in (8). Laparatomy (LP) was carried out as a control for surgical stress. At various times after PH or LP (6, 12 and 24 h) rats were sacrified and the remnant liver removed.

Obtention of nuclei and nuclear fractions. Hepatocyte nuclei and nuclear subfractions were obtained according to (9). Briefly, purified nuclei were resuspended in buffer A (250 mM sucrose, 5 mM $MgSO_4$, 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.5 μg/ml aprotin and 50 mM Tris-HCl, pH 7.4) containing 250 μg/ml DNase I plus 250 μg/ml RNase A. After 1 h incubation at 4°C, nuclei were sedimented at 800 × g for 10 min. The supernatant was collected and named S1 and the pellet was resuspended in 10 mM Tris-HCl pH 7.4, 0.2 mM $MgSO_4$, 1 mM PMSF and 0.3 μg/ml aprotinin (buffer B). Buffer B containing 2 M NaCl was then slowly added to a final NaCl concentration of 1.6 M and incubated during 15 min. Then, the samples were centrifuged at 5,000 × *g* for 20 min and the supernatant (S2 fraction) and the pellet (nuclear matrix) were collected.

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Phosphorylation assays. The determination of CK2 activity in the S1, S2 or nuclear matrix fractions was performed as previously described (10).

Alternatively, the presence of CK2 activity in the S1 and S2 fractions (10 μg) from control livers and at different times after PH or LP was detected as described in (11) with slight modifications, which consisted in using [γ-^{32}P] GTP as phosphate donnor and 0.5 μg of β-casein as substrate. The phosphorylation reactions were subjected to electrophoresis and autoradiography as previously described (11). The phosphorylated β-casein was quantified using the BioImage System (Millipore). To detect the presence and phosphorylation of endogenous substrates the same reaction was performed but in the absence of β-casein.

Immunoblotting. Western blot experiments were carried out as previously described (11) using affinity purified polyclonal antibodies against the CK2α subunit (Upstate Biotechnology Inc., USA) (1:500 dilution) or affinity purified antibodies (1:500 dilution) raised against a peptide based on the sequence of CK2β according to (12). The bands were quantified using the BioImage System (Millipore).

Protein determination. The protein content of the samples was estimated by the method of Bradford (13), using bovine serum albumin as a standard.

RESULTS

In order to analyze the intranuclear distribution of CK2, three nuclear fractions (S1, S2 and nuclear matrix) were obtained from rat liver cells and the kinase activity measured in all of them. The specific activity was higher in the S2 fraction (0.118 ± 0.023 U/mg protein) than in S1 (0.061 ± 0.005 U/mg protein) and nuclear matrix (0.022 ± 0.005 U/mg protein). Western blot analysis using specific antibodies against CK2 α and β subunits were also carried out in these three nuclear fractions. Results revealed that the anti-α subunit antibodies recognized three bands in the S1 and S2 fractions (fig. 1) whereas the reaction in the nuclear matrix was very weak (data not shown). These bands were named as α_1, α_2 and α_3, from high to low Mr. The α_1 and α_2 bands behaved as the classical α and α' isoforms as it can be seen when compared with the purified enzyme from the rat liver cytosol, which comprises only these two isoforms (fig. 1). The third band α_3 could be a degradation product of α or α' subunits, a modification of them or a non-described nuclear isoform. Interestingly, the major band in S1 is α' whereas α predominates in the S2 fraction. The antibodies against the β subunit only recognized a single band of 28 kDa in all the cases. To analyze possible modifications in the amount of the different nuclear CK2 subunits during regeneration, western blot analysis was carried out on nuclear fractions from rat liver cells obtained at different times after a PH or a LP. We selected three times after hepatectomy corresponding to the early G_1 (6 h), late G_1 (12 h) and S phase (24 h) (14). Fig. 2 shows that at all the studied times a pattern of three bands was observed in S1 although the intensity of α_3 was highly increased at 6 h in both regenerating and sham-operated rats. Likewise, the α_1 (α) subunit was significantly decreased at this time after PH or LP. In S2 samples the three bands were also observed at all the studied times although no major modifications of the pattern were observed during regeneration or after LP. The quantification of the amount of the different catalytic subunits in the S1 fractions revealed significant increases of α_2 (1.9 fold) and α_3 (4.5 fold) subunits at 6 h after PH (table I). In laparatomized animals also a significant increase of α_3 (3 fold) was found at 6 h, although the elevation was lower than in hepatectomized animals. Moreover, α_1 (α) was decreased (about 50 %) at 6 h after PH or

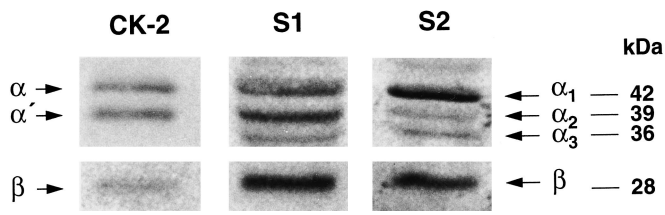

FIG. 1. Localization of CK2 in nuclear subfractions. S1, S2 samples and purified CK2 from cytosol (CK2) were analyzed by western blotting using specific antibodies against the α and β CK2 subunits. The anti-α antibodies recognize three bands (α_1, α_2 and α_3) in both nuclear samples. The α_1 and α_2 bands correspond to the α and α' isoforms of the purified enzyme.

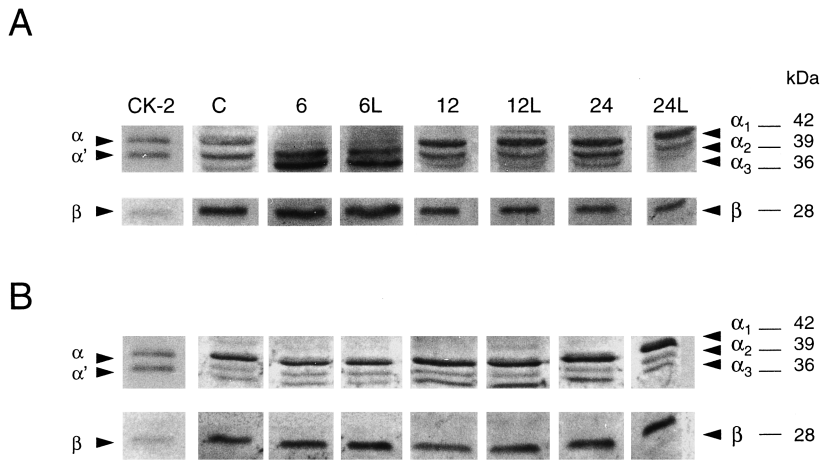

FIG. 2. CK2 in S1 and S2 nuclear fractions during liver regeneration. S1 (A) and S2 (B) nuclear fractions obtained from non-operated rats (C) and at different times after a PH (6,12 and 24 h) or after a LP (6L, 12L, 24L) and CK2 purified from cytosol were analyzed by western blotting using specific antibodies against the α and β CK2 subunits. The anti-α antibodies recognize three bands (α_1, α_2 and α_3) in both nuclear samples. α_1, α_2 correspond to the α and α' isoforms of the purified enzyme.

LP. At 12 h no significant modifications in the amount of CK2 were found whereas at 24 h slight decreases of α_3 and increases of α_1 (α) were observed (table I). Interestingly, the amount of CK2 β subunit was progressively decreasing from 12 h after surgery in the S1 fractions.

The activity of CK2 was measured in S1 and S2 nuclear fractions after PH or LP. As shown in fig. 3 the activity of CK2 in the S1 fraction increased at 6 h after PH (5.5 fold) respect to non-operated control rats. Sham-operated rats also showed an increase of CK2 activity at 6 h although in this case it was significantly lower than in hepatectomized rats (2.5 fold). At 12 h after PH or LP CK2 activity was very low, even lower than in non-operated control rats. Finally, at 24 h a slight increase of CK2 activity was found in hepatectomized (1.9 fold) but not in sham-operated rats. CK2 activity was also measured in S2 fractions but only minor changes were observed at 6 and 24 h post-hepatectomy (data not shown).

The ratio β/α_T ($\alpha_T = \alpha_1 + \alpha_2 + \alpha_3$) was also studied in S1 fractions during liver regeneration in order to analyze whether CK2 activity correlated with changes in the subunit structure of the enzyme. The relation α/α':β is 1:1 in the preparation of enzyme purified from rat liver cytosol. Thus, we used purified enzyme as a control for the quantification of the β/α_T ratio at each time point. In the S1 fraction of normal non-operated liver the β subunit was 2.8 fold more abundant than the catalitic subunits. At 6 h the ratio was around 2 in both hepatectomized or laparatomized

TABLE I
Amount of Each CK2 Subunit at Different Times after PH or LP in S1 Nuclear Fraction

	α_1 (α)	α_2 (α')	α_3	β
Control	100	100	100	100
6h	52.5	190.5	450	138
6hL	57.5	123	315.5	95.5
12h	126.5	105	81	61
12hL	167.5	93.5	68	54
24h	146	90.5	78	49.5
24hL	120	51	31	31

Values are expressed as percentage respect to control. In all the cases the standard deviation was less than 15%. Laparatomy samples are denoted by "L."

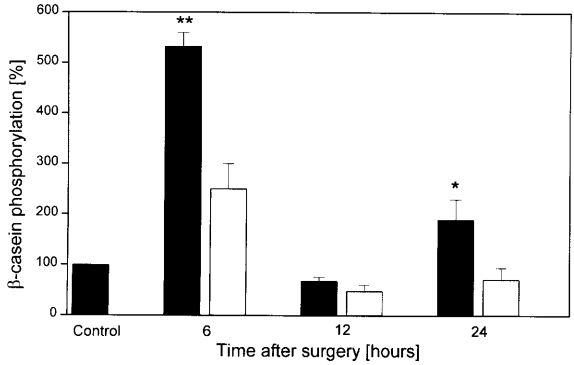

FIG. 3. CK2 activity in the S1 nuclear fraction during liver regeneration. CK2 activity was measured in the S1 fraction obtained from non-operated rats (control) and at 6, 12 and 24 h after a PH (black bars) or LP (white bars). Values represent the means, of at least three experiments ± s.e.** ($p \leq 0.001$), *($p \leq 0.005$).

samples and then the values were progressively decreasing becoming around 1 at 24 h after PH or LP. These results suggest that CK2 activity was not related to the ratio β/α_T.

With the aim to identify nuclear substrates of CK2 which could appear during liver regeneration, endogenous phosphorylation experiments were performed in S1 fractions. As it can be seen in fig. 4 six major proteins were phosphorylated by CK2 in these fractions. Two of these substrates have been identified as the heterogenous nuclear ribonucleoproteins (hnRNP) A2 and C by western blotting (data not shown) whereas the other four (p80, p34, p28 and p25) still remain unknown. Results did not reveal the phosphorylation of new proteins neither after PH nor after LP, although quantitative changes could be observed, the phosphorylation of some bands increased whereas in other cases the phosphorylation was decreased after surgery.

DISCUSSION

Two major pools of CK2 exist in the nucleus of rat liver cells: one can be released by the action of nucleases and the other one with the subsequent treatment with 1.6 M NaCl. The specific activity in the latter fraction (S2) is about 2 fold higher than in the former (S1). Since the nuclease treatment mainly releases from the nuclei the proteins associated with the hnRNA located at the transcription sites or with the hnRNPs, the results suggest that CK2 is associated with the transcriptionally active chromatin and in consequence it could be involved in transcription and/or RNA processing (15).

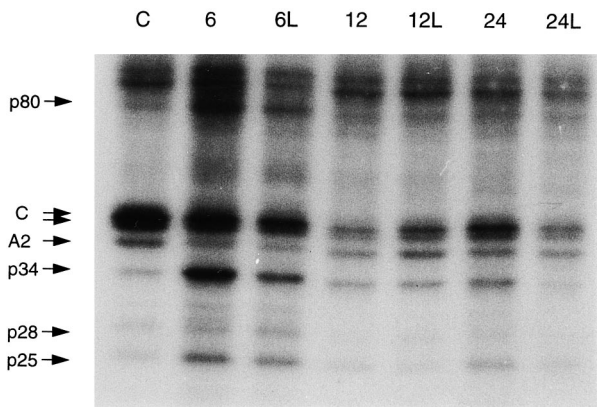

FIG. 4. CK2 substrates in the S1 fraction during liver regeneration. Endogenous phosphorylation experiments were carried out in S1 samples obtained from non-operated rats (C) and at 6, 12 and 24 h after a PH (numbers) or LP (numbers marked with "L"). The apparent Mr of the phosphorylated proteins are labeled at the left side of the autoradiography. C and A2 correspond to the hnRNP proteins C and A2, respectively.

These results are in agreement with the fact that a number of CK2 substrates are transcription factors or hnRNA associated proteins (1,4,15). The second pool of CK2 is weakly associated to the nuclear matrix since it can be released from this structure with high salt containing buffers. The functional significance of this CK2 extracted with high salt remains to be established.

The catalitic subunits are differentially distributed in the nuclear subfractions: the α' isoform is mainly associated to the S1 fraction whereas the α is present in both S1 and S2 fractions although it is enriched in the latter fraction. These results suggest that specific substrates would be phosphorylated by each isoform at different nuclear locations.

Results also indicate that CK2 is activated during proliferation of rat liver cells in a biphasic manner in the S1 fraction but not in fraction S2. The first peak occurs during early G_1 (6 h after PH). This is in agreement with a previous work which revealed an increase in CK2 activity in total liver extracts at 6 h after PH (16). Although an increase of CK2 activity in S1 fraction was also observed at 6 h after a LP, in this case the activity was significantly lower than that generated by PH indicating that a specific increase in CK2 activity is generated during proliferation. This peak of CK2 activity could be involved in the elevation of the transcriptional activity observed during G1 in regenerating liver cells (17). The second peak of CK2 activity which occurs at 24 h after PH is much lower than that observed at 6 h and can be involved in DNA replication since recent reports have involved CK2 in the triggering of DNA synthesis (18). The measurements of the ratio β/α_T after PH or LP indicate that the relative amounts of the α and β subunits in the S1 fraction are not related to CK2 activity since the same ratio is observed in quiescent cells and at 6 h after PH or LP whereas the activity at these times is quite different.

Interestingly, the increase of CK2 activity in S1 fraction at 6 h after PH or LP correlates with the increase of the α_3 isoform. This fact supports that α_3 could be a highly active isoform of CK2. This possibility is under investigation in our laboratory. The increase of CK2 activity at 24 h after PH cannot be attributed to the elevation of the amount of the α_3 isoform. However, since the levels of α_1 were increased at this time after PH, the increase of α_1 could be responsible for this peak of CK2 activity.

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