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DEXAMETHASONE INDUCED CHANGES IN DNA LIGASES IN CHICK EMBRYO THYMUS

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Summary

When injected into the egg at the 16th day of incubation, dexamethasone was found to produce in thymus a complete replacement of the 8.2 S form of DNA ligase by the 6.2 S form, with a 8-fold increase in activity. A decrease in α- and γ-DNA polymerase activity was observed while β-DNA polymerase was found to be unaffected.

INTRODUCTION

After its discovery in bacteria and phage infected bacteria, DNA ligase has been studied in various eukaryotic systems (1). In calf thymus, two different forms of DNA ligase, not immunologically related have been purified (2). In 9 day whole chick embryo, two DNA ligases are present, a main enzyme (8.2 S) representing more than 90 % of the total ligase activity and a minor 6.2 S enzyme (3, 4). In the chick embryo thymus, only the 8.2 S enzyme can be found until birth, whereas the 6.2 S form represents the unique activity for the chicken after hatching (5). The reasons and mechanisms of this birth change are not clear. Since the thymus is known to respond drastically to corticosteroids by cellular changes (6, 7, 8) and modification of the DNA metabolism (9), we decided to investigate possible changes of DNA ligases under the effect of dexamethasone. We also examined the variations of activity of the three known DNA polymerase, α , β , and γ, involved with the DNA ligases in the processes of DNA replication and/or repair.

MATERIAL AND METHODS

Dexamethasone administration. White Leghorn eggs incubated at 37° ± 1° C were injected on the 16th day with 10 µM of dexamethasone phosphate (Merck) in 0.5 ml of saline (8°/₀₀ NaCl) into the air chamber, or with saline only as control. After two more days of incubation (day 18), the embryos were removed and thymus dissected as previously described (5).

Preparation of extracts for sucrose sedimentation. 0.200 g of thymus from control or dexamethasone treated 18 day old chick embryos were homogeneised with a Sorvall Omni Mixer (teflon pestle) in 3 volumes of extraction buffer

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(0.5 M KCl, 20 mM Tris-HCl, pH 7.4, 2 mM dithiothreitol and 0.2 % NP 40) made 0.8 mM in PMSF (phenylmethyl-sulfonyl fluoride, Sigma) just before use. The homogeneisation was followed by a sonication (Alcatel Sonicator, France) set on 2.2 during three periods of 20 s separated by 20 s intervals and by a 140 000 x g centrifugation for 60 min. The supernatant was made to correspond to 100 mg/ml of protein, measured according to Lowry et al. (10). 250 µl of this extract (equivalent to 4 glands from the controls and 14 glands from treated animals) were layered on the top of sucrose gradients 5-20 % in 0.5 M NaCl, 50 mM Tris HCl pH 7.4, 1 mM EDTA and 2 mM dithiothreitol, and centrifuged for 15 hours at 47 000 rpm in a SW 50 rotor at 4° C.

Preparation of extracts for chromatography on phosphocellulose. Thymus were homogeneised as described above but the protein content of each extract was 100 mg, corresponding to 16 glands for control and 40 glands for dexamethasone treated animals. This extract was dialyzed overnight against a buffer containing 20 % glycerol, 50 mM Tris-HCl pH 7.4, 1 mM 2-mercaptoethanol, 0.1 mM EDTA and 50 mM KCl (TEMG buffer). The extract was adsorbed on a phosphocellulose (P11 Whatmann) column (0.5 x 8 cm) previously equilibrated in the same buffer and the enzyme eluted by a linear gradient from 0.1 M to 0.7 M KCl in TEMG buffer.

Enzyme assays.

- DNA ligases assay : DNA ligase was tested after a modification (5) of the method described by Olivera (11) : after dephosphorylation by alkaline phosphatase (Sigma), oligo dT_{12-18} (PL Biochemicals) was rephosphorylated in the presence of [^{32}P] ATP (Amersham, England) with polynucleotide kinase (Boehringer). For the ligase activity determination, each assay (300 µl) contained 6 nmol. of oligo dT_{12-18}, 6nmol. of Poly-dA (PL Biochemicals, Milwaukee), 25 mM Tris HCl, pH 7.6, 7.5 mM $MgCl_2$, 1 mM ATP, 1 mM dithiothreitol, 10 µg of bovine serum albumin and 40 µl of the enzyme. The assays were incubated for 30 min at 37° C, then diluted twice with water and incubated at 80° C for 30 min. 0.015 units of alkaline phosphatase were added at time 0, and 0.0075 units at time 10 and 20 min. At the end of the incubation, the assays were TCA precipitated (5 % final), filtered through Millipore filters, the filters were dried and the radioactivity measured. One unit of DNA ligase is defined as the activity which renders 1 mumole of [^{32}P] oligo dT resistant to alkaline phosphatase in 30 min.

- DNA polymerase assay : The activity of the DNA polymerases was determined on aliquots of fractions from the sucrose gradients used for the DNA ligase determination, using the following conditions.

α -polymerase : The reaction mixture (0.1 ml) contained 50 mM Tris-HCl, pH 7.4, 8 mM $MgCl_2$, 16 mM dithiothreitol, 0.1 mM each dATP, dCTP and dGTP, 0.03 mM ^{3}H -dTTP (333 mCi/mmol), 200 µg/ml bovine serum albumin, 120 µg/ml activated calf thymus DNA (prepared by pancreatic DNAse partial digestion according to Aposhian and Kornberg, (12) and 10 µl of enzyme fraction. Incubation was at 37° C for 60 min. TCA insoluble radioactivity was measured.

β -polymerase : The enzyme (15 µl) was preincubated at 0° C for 30 min with 5 mM N-ethylmaleimide. To measure the activity, the reaction mixture contained in a final volume of 0.1 ml : 50 mM Tris HCl, pH 8.2, 75 mM NaCl, 8 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 0.1 mM each dATP, dCTP and dGTP, 0.03 mM [^{3}H]-dTTP (667 mCi/mmol), 200 µg/ml bovine serum albumin and 120 µg/ml activated calf thymus DNA. Incubation was for 45 min at 37° C. TCA insoluble radioactivity was measured.

γ -polymerase : The reaction mixture (0.1 ml) contained 25 mM Tris-HCl, pH 8.2, 50 mM K phosphate buffer, pH 8.4, 0.5 mM $MnCl_2$, 2 mM dithiothreitol, 75 mM KCl, 0.0071 mM ^{3}H -dTTP (2667 mCi/mmol), 200 µg/ml bovine serum albumin and 8.0 µg/ml poly rA : $dT_{(12-18)}$ (10 : 1) (Poly rA, from Miles, and

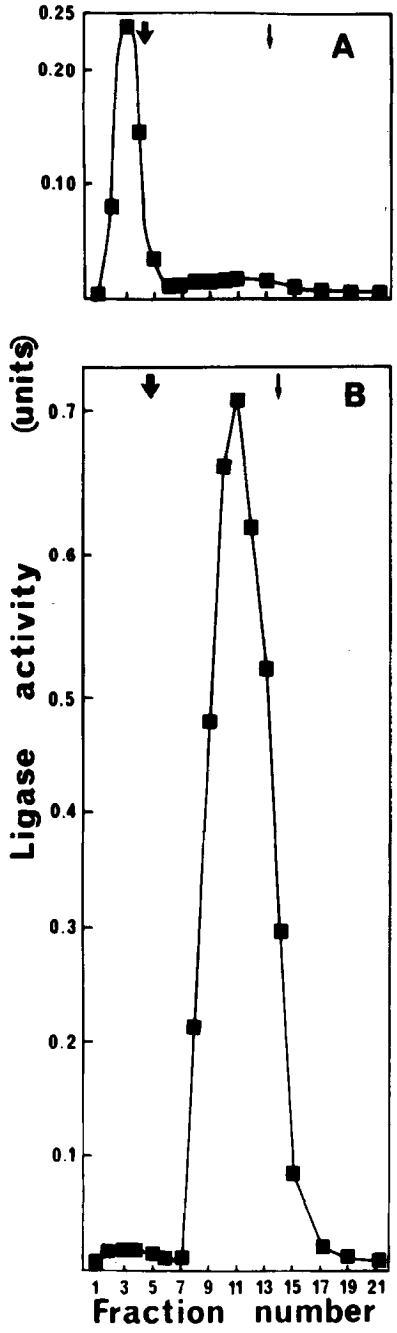

Figure 1 : Effect of dexamethasone on the sedimentation behavior of thymic DNA ligases from chick embryo.
Thymic extracts were made as described in Material and Methods. Fractions of 200 µl were collected and 40 µl aliquots assayed for enzyme activity. Fig. 1_A is the sucrose gradient behavior of the control extract and Fig. 2_A is that of the thymic extract from dexamethasone injected eggs. Centrifugation is from right (top) to left (bottom). Arrows indicate the position of DNA polymerase α (7.4 S) (thick arrow) and DNA polymerase β (3S) (thin arrow).

oligo $dT_{(12-18)}$, from Pl-Biochemicals, were hybridized at 70° C) and 5 µl of enzyme fraction. Incubation was for 60 min at 37° C. TCA insoluble radioactivity was measured.

RESULTS AND DISCUSSION

Depicted in Fig. 1 is the centrifugation pattern on sucrose gradients of DNA ligase activity of a thymic extract from control (1_A) and dexamethasone injected eggs (1_B). In control thymus extract, only one peak of DNA ligase activity, corresponding to the 8.2 S form, is present. In contrast, in the dexamethasone treated embryo thymus extract, this form is absent and replaced by the 6.2 S form of DNA ligase. Furthermore the activity of DNA ligase in thymus extract of treated embryos is enhanced by a factor 8.

Shown in Fig. 2_A is the result of a phosphocellulose chromatography of thymic extract from 16 day untreated embryos. The major peak of DNA ligase activity was eluted with a KCl molarity of 0.55. The control extract from 18 day embryo thymus also shows a major peak of activity for 0.55 M KCl (Fig. 2_B). In the thymic extract of dexamethasone treated embryos, only one peak of ligase activity, eluted at a KCl molarity of 0.35, is present (Fig. 2_C) and the total activity is increased to the same extent as found in the analysis on sucrose gradient.

These observations are in strong support to a change of DNA ligase form in the chick thymus under the influence of dexamethasone. However there could be a direct effect of dexamethasone on DNA ligase activity. To check this point, dexamethasone was added *in vitro* to the thymic extract from 18 day control embryos to a final 1 mM concentration and allowed to react for 30 min at room temperature. After centrifugation analysis the ligase activity was still present only in one peak (8.2 S) without increase as compared to the extract without dexamethasone. Other control experiments were performed by mixing aliquots of extracts from thymus dissected from embryos which had received dexamethasone or not. No modification in the migration and intensities of the peaks was found. This observation rules out a possible inhibiting or converting intrinsic factor.

To check for the possible existence of a dimer-monomer equilibrium between the two forms of ligase, thymic extracts from dexamethasone treated and control animals were run in sucrose gradients containing 6M urea. The fractions were dialyzed against 50 mM Tris HCl buffer (pH 7.4) containing 1 mM 2-mercaptoethanol and 0.1 mM EDTA and assayed for DNA ligase activity. No modification of the sedimentation pattern of the two forms of enzyme was observed under these conditions, excluding the hypothesis of a simple dimer-

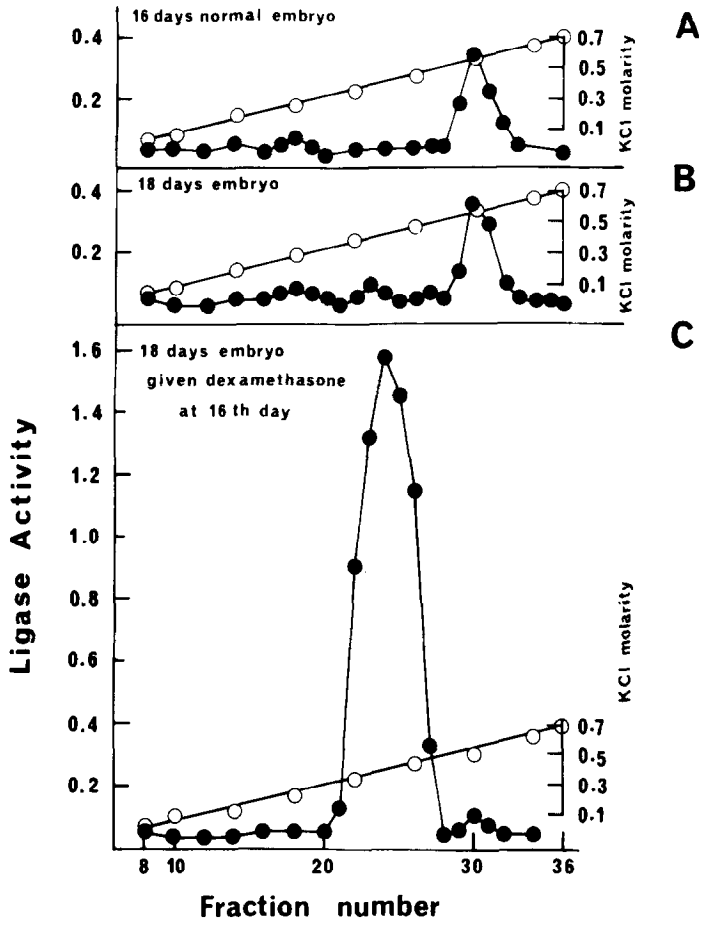

Figure 2 : Effect of dexamethasone on the behavior of thymic DNA ligase from chick embryo on phosphocellulose.
Thymic extracts from 16 day untreated embryos (Fig. 2_A), 18 day controls (Fig. 2_B) and 18 day chick embryos which have received dexamethasone at 16 days (Fig. 2_C) were made as described under Material and Methods. Fractions of 1.5 ml were collected and 40 µl aliquots were assayed for DNA ligase activity.

monomer conversion. This result, which has also been obtained with the 8.2 S and 6.2 S forms observed in the course of normal development of the thymus (5), rules out a possible modification of the sedimentation properties of DNA ligase by interaction with other cellular components present in the unpurified extract analysed on the gradient. Moreover, the thermal stability of the two forms is different : the 8.2 S form was found to be 50 % inactivated by 10 min heating at 52° C, while the 6.2 S enzyme is 50 % inactivated by 10 min heating &t 43° C. These observations are in favor of the existence of two different DNA ligases entities. A similar replacement of the 8.2 S from by a 6.2 S form of DNA ligase has been observed in normal development of chick thymus at the time of hatching (5).

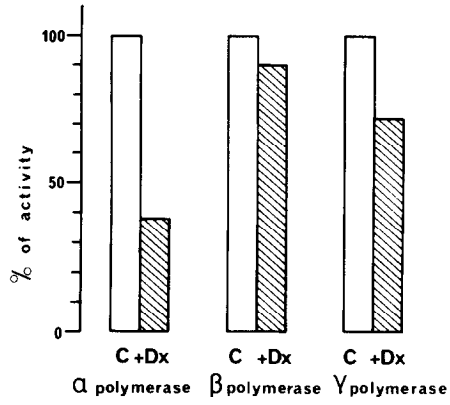

Figure 3 : Effect of dexamethasone on DNA polymerase α , β and γ activities in 18 day old chick embryo thymus.
The activity of the DNA polymerases in the extract from dexamethasone treated 18 day old chick embryo thymus (+ Dx) is expressed as compared to the activity in the 18 day control thymus extract (C), taken as 100 %. Since the same amounts of proteins were put on the two gradients the comparison involves activities expressed per weight of thymic proteins.

DNA polymerase α , β and γ activities were determined on the previous sucrose gradients using different templates and specific incubation conditions (13, 14). As shown in Fig. 3, DNA polymerase α presents a 62 % decrease in activity at 18 days, after dexamethasone treatment, as compared with the extract from untreated chick embryo thymus. DNA polymerase γ activity decreases by 28 %, while DNA polymerase β remains almost constant. No major modification of the sedimentation patterns of either of these DNA polymerases was observed. These results are comparable to what has been described for mouse thymus after 24 hours cortisone treatment (15).

Since the changes observed here in DNA ligases, under the effect of dexamethasone, at 18 days are observed in normal development of the thymus in a later stage, we can conclude that, at this level, dexamethasone produces developmental changes which would normally occur in the thymus only after hatching. To our knowledge this is the first report describing a direct steroid induced change of molecular forms of DNA ligases in thymus.

Since hormones like cortisone are known to enrich the thymus in immuno-competent cells (6, 16, 15), it is not unlikely that the appearance to the 6.2 S DNA ligase or its increased activity, as well as the variations in the levels of DNA polymerases α and γ are correlated with this enrichment. Investigations are now in progress in attempt to correlate these enzymatic changes with changes in the types of cells in the thymus and to check if they are restricted to this organ.

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