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Communication

Effect of protein kinase ck2 on topoisomerase I from plasmodia of the slime mold *Physarum polycephalum*[°]

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Relaxing activity of *Physarum* topoisomerase I was increased by calf thymus protein kinase ck2, similarly as was the activity of mammalian topoisomerase I, despite a pronounced difference between amino-acid sequences of non-conserved domains of *Physarum* and mammalian enzymes. This feature of *Physarum* topoisomerase I was cancelled in nuclear extracts isolated from dibutyryl-cAMP treated plasmodia in which the activity of protein kinase ck2 was elevated.

Eukaryotic DNA topoisomerase I (topo I, EC 5.99.1.2) is an abundant topoisomerase which participates in numerous DNA transactions (recent review: [1]). It is a housekeeping enzyme the activity of which is usually maintained at a fixed level. Several mechanisms have been revealed which might regulate topo I activity in the cell [2]. Stimulation of the expression of the *TOP1* gene in mammalian cells has been shown to occur upon treatment with phorbol esters [3], serum [4] and EGF [2]. However, the level of *top1*mRNA does not seem to be a critical factor for the relaxing activity of the enzyme [2, 4, 5]. Topo I protein may undergo two posttranslational modifications that affect its enzymatic activity. Poly(ADP-ribosylation) of the enzyme leads to inhibition [6] whereas phosphorylation of topo I results in an increase of the relaxing activity [7–9].

A special feature of topo I is its ability to form with numerous proteins complexes in which the relaxing activity of topo I is significantly elevated. One of the proteins that form

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Abbreviations: ck2, protein kinase ck2; db-cAMP, dibutyryl-cAMP; EGF, epidermal growth factor; topo I, topoisomerase I.

complexes with topo I considered to be a possible regulator of activity, is protein kinase ck2 [10, 11]. However, no evidence indicating that such a mechanism is active *in vivo* has been presented.

In the previous work we observed a fourfold transient increase of topo I activity in starved plasmodia of *Physarum polycephalum* induced to sporulate by light impulses [12]. Transduction of the light-induced signal in sporulating plasmodia is linked with changes of internal cAMP level [13–15]. We demonstrated that the effect of light on topo I activity could be mimicked in the dark by db-cAMP administered to plasmodia [12]. Looking for the mechanism of the *Physarum* specific activation of topo I by cAMP we excluded an increase of *top1*mRNA as a possible reason [16]. In this communication we present evidence suggesting participation of ck2 in the activation of topo I by cAMP.

MATERIALS AND METHODS

Culture. *P. polycephalum*, strain CL, was obtained from Department of Genetics, Leicester University (U.K.). Because plasmodia of *P. polycephalum* significantly change their basic properties upon prolonged culture in a liquid medium [17, 18], the cultures were renewed every six months. Microplasmodia were grown in shaken cultures at 23°C in a semidefined medium [19]. Fresh cultures (24 h after inoculation) were transferred into starvation medium [19]. db-cAMP was added directly to the culture medium.

Nuclear extracts. Nuclear extracts were isolated according to the protocol described previously which included a step of hydroxyapatite chromatography [20].

Mouse topo I. Mouse topo I was isolated from mouse lymphoma L5178Y-R cells as described previously [5].

ck2. ck2 was isolated from calf thymus according to Mills *et al.* [21]. No DNA relaxing activity was present in the preparation. SDS/PAGE revealed bands corresponding to the kinase subunits as a predominant component of the preparation.

Enzymatic assays. Topo I assay measured the relaxation of supercoiled pBR322 according to Liu [22]. One unit relaxed 50% of the substrate DNA after 30 min at 25°C. When determined in *Physarum* nuclear extracts, topo I activity was referred to DNA content in the nuclei. ck2 Assay measured the radioactivity introduced into casein from [γ-^{32}P]ATP according to Glover & Allis [23]. One unit represented the amount of enzyme transferring 1 pmol of ^{32}P to casein/min [23]. Protein kinase C activity was determined using PepTag Assay (Serva).

Electrophoresis. Electrophoresis of DNA was performed according to Maniatis *et al.* [24]. Electrophoregrams were photographed and negatives were scanned by densitometry.

RESULTS AND DISCUSSION

Relaxing activity of *Physarum* topo I was stimulated by addition of calf thymus ck2 to the extent similar as for mouse topo I (Table 1). This was observed despite a pronounced difference in amino-acid sequences between the non-conserved domains of *Physarum* and mammalian topo I [16]. Of four distinct domains of topo I polypeptide only two are conserved and vital for the relaxation re-

Table 1. Effect of calf thymus ck2 on the relaxing activity of mouse and *Physarum* topo I.

Calf thymus ck2 was added in an amount corresponding to 5 activity units per 1 activity unit of topo I. *Physarum* topo I was isolated from plasmodia cultured without db-cAMP. Results are mean values ± S.D. from 3 experiments.

Topoisomerase I	Increase upon ck2 treatment*
Mouse	3.84 ± 0.35
Physarum	4.30 ± 0.51

*Relaxing activity determined in the absence of ck2 was assumed to be 1.

action [25, 26]. The non-conserved N-terminal domain is the fragment of the polypeptide in which the specific interaction between topo I and another protein(s) has been localized [27, 28]. Comparison of amino-acid sequences of *Physarum* and mouse N-terminal domains is shown in Fig. 1.

ck2 had no effect on topo I activity in the nuclear extracts from plasmodia treated with 100 μM db-cAMP (Fig. 2). When referred to DNA content in the nuclei, the relaxing activity in nuclear extracts from db-cAMP treated plasmodia was 4–5-fold higher than that determined for the control plasmodia. This suggested that a similar extent of activation of topo I could result either from ck2 added to the extract or from an unidentified factor that appeared in the nuclei upon db-cAMP treatment of the plasmodia. To test the possiblity that the factor was *Physarum* ck2 we compared patterns of topo I and ck2 activities in db-cAMP treated plasmodia.

Determinations of protein kinases activities in *Physarum* nuclear extracts were preceded by treatment of the extracts with hydroxyapatite to remove an acidic polymer [29] that affected ck2 activity. We detected no protein kinase C activity in the extracts (not shown). On the other hand, ck2 activity was at the range of 0.5–1.2 pmol of ^{32}P incorporated into casein/min per mg nuclear protein. The pattern of ck2 activity in db-cAMP treated plasmodia closely resembled that of topo I activity. Both activities reached a maximum level at 4 h after addition of db-cAMP to the concentration of 100 μM (Fig. 3). 100 μM 5,6-dibromo-1-(β-D-ribofuranosyl)benzimidazole (DiBr-DRB), which is an inhibitor specific for ck2 [30], inhibited more than 90% of ^{32}P incorporation into casein catalysed by *Physarum* nuclear extracts. The remaining small ^{32}P incorporation into casein showed no changes upon db-cAMP treatment.

The above observations suggest that ck2 might be a direct reason of the cAMP-induced increase of topo I activity in *Physarum* plasmodia. Unknown remains the pathway between plasmodial target for db-cAMP and ck2. Early works on *Physarum* reported the presence of cAMP-inhibited casein kinase in the plasmo-

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mus M-------SGDHLHN-------------------------DSQIEADFRLND------------

    *       .*  .                            * . . *. * .

mus ------------------------------------------------------------------
phy TPTTSKPAATTPKPAATTSKPASTTPKPAAAIPKSPAKPAATTPKPAAAATGKPAASPSTAKPAA


phy TTSKPAASPAKPAGTAKPAGTAKPAATTPKPAAGAAKPASSTPKPAAGAAKPAATTPKPAGNAND
                  . *  .  *      *             .* . .  * .    *   ....

mus KDGSSEKHKDKHKDRDKERRKEEKIRAA----------GDAKIKKEKENGFSSPPRIKDDPEDDG
phy NKDKVEKTTDTTIETTTTPKATQSTSTTVTTTKTNTPTASSKVTVTNTLTTTTPTKTNDDSDDDK


mus YFAP-------------------------------------PKEDIK----------PLKRLRD
phy PLSSLISEKKVTVVKKEKKDDEIVTTAKTTTVKKEVKKEDTAKKDVKKEVKKETPKKTPAKRKAA
     ...                                      .* *.*           * **

mus E---DDADYKPK-KIKTEDIKKEKKRKSEEEEDGKLKKPKNKDKDK-----KVAEPDNKKKKPKK
phy ESSSEESDFSEDEKPKKKAAKKKKKSDSDESDDEPKKKATTKAKANPKKEVKEEGGEAKKKGKKK
    *   ...*.    * *    ** **  *.* .*   **.  * * .      *    . *** **
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Figure 1. Comparison of amino-acid sequences of the N-terminal domains of mouse (mus) and *Physarum* (phy) topo I.

GenBank accession numbers were: X83758 for the mouse and U63217 for *Physarum* topo I sequences. *, sites for identical amino-acids.

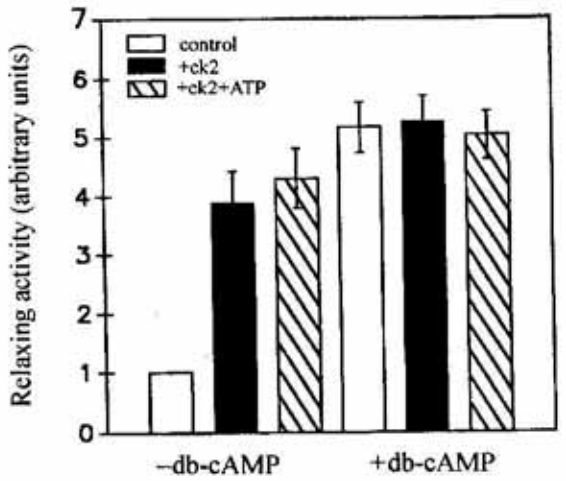

Figure 2. Relaxing activity in nuclear extracts from plasmodia, determined in the presence of ck2.

db-cAMP treated plasmodia were cultured in the medium containing 100 μM nucleotide for 4 h. Calf thymus ck2 was added in an amount corresponding to 5 activity units per 1 activity unit of topo I. 5 μM ATP was added where indicated. Relaxing activity was referred to DNA content in the isolated nuclei. Relaxing activity in extracts from plasmodia not treated with db-cAMP, determined in the absence of ck2, was assumed to be 1. Results are mean values ± S.D. from 3 experiments.

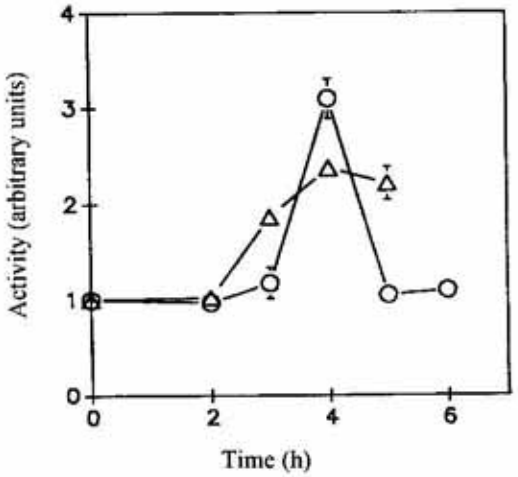

Figure 3. Time course of changes of topo I and ck2 activities measured in nuclear extracts isolated from plasmodia treated with 100 μM db-cAMP.

The activities were referred to DNA content in the isolated nuclei. The values for plasmodia cultured in the growth medium were assumed to be 1. ○, Topo I; △, ck2. Results are mean values ± S.D. from 4 experiments.

dia [31]. We observed neither an inhibitory nor a stimulatory effect on topo I activity of cAMP added directly to the nuclear extracts, even when the latter were enriched in purified protein kinase A up to the ratio of 10 activity units of the kinase per 1 activity unit of topo I (not shown).

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