# DIMETHYL SULPHOXIDE STIMULATES THE PHOSPHORYLATION OF TWO MAJOR DNA BINDING PROTEINS OF YOSHIDA ASCITES TUMOR CELLS

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## ABSTRACT

Yoshida ascites tumor cells contain two major DNA-binding proteins (DBP) of molecular weight 34,000 and 38,000 daltons. [$^{32}$P] phosphate incorporation studies indicate that these are the major phosphoproteins in addition to a 115,000 dalton DBP. Dimethyl sulphoxide (DMSO) treatment of the tumor cells for 8 hr resulted in a six-fold stimulation of the phosphorylation of the 34 KD DNA-binding protein. Under the same conditions, DMSO stimulated the phosphorylation of the 38 KD DBP about two fold. The phosphorylation of the 115 KD DBP was inhibited by DMSO.

## INTRODUCTION

We have recently identified and purified a 34,000 dalton DNA-binding protein (DBP) from Yoshida ascites sarcoma cells[1,2]. This protein has been shown to be phosphorylated and the extent of phosphorylation altered the binding characteristics of the protein to DNA[1]. The purified DBP was shown to possess endonuclease activity capable of making a single cut in one of the strands of pBR 322 DNA[2]. Here, we show that DMSO which is known to specifically stimulate tyrosine residue phosphorylation[3] stimulates phosphorylation of the 34 KD DBP in Yoshida ascites tumor cells. Another DNA-binding protein, the topoisomerase has been shown to be capable of tyrosine residue phosphorylation[4].

## MATERIALS AND METHODS

Yoshida ascites tumor cells were propagated as described earlier[1]. For phosphorylation studies, 5 mCi of carrier-free [$^{32}$P] phosphate was injected intraperitoneally to rats bearing the tumor in the presence or absence of 10% DMSO. Cells were harvested after 8 hr and washed with PBS. Details of preparation of 30,000 g supernatant (S-30), DNA-cellulose chromatography of the cell extracts, polyacrylamide gel electrophoresis of the eluted proteins and autoradiography have been described earlier[1]. In this study, DNA-binding proteins were isolated by affinity chromatography on denatured calf-thymus DNA-cellulose columns[1].

## RESULTS AND DISCUSSION

Figure 1 shows that a protein of molecular weight 34,000 dalton is the major DNA-binding protein of Yoshida ascites sarcoma cells. The steady-state level of this protein was not increased by DMSO treatment (compare lanes B and D). DMSO treatment did not result in the alteration of the content of most of the

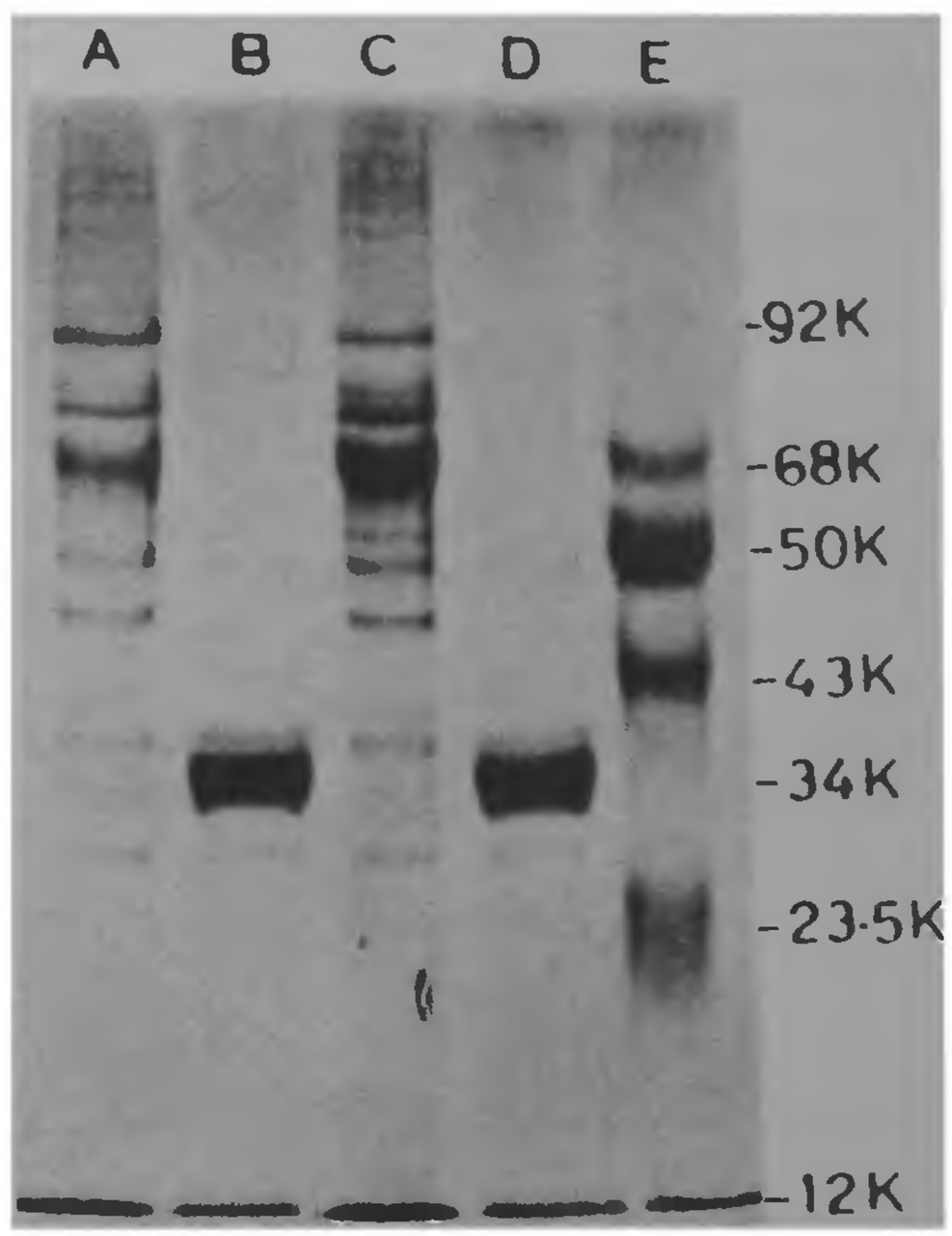


**Figure 1.** Coomassie Blue staining profile of the DNA-binding proteins of DMSO treated and control cells. **A.** control S-30; **B.** DNA-binding proteins from S-30 of untreated cells; **C.** S-30 from DMSO treated cells; **D.** DNA-binding proteins from S-30 of DMSO treated cells. Lane **E** shows molecular weight marker proteins. The proteins were electrophoresed in 10% polyacrylamide gels containing sodium dodecyl sulphate (SDS).

cytoplasmic proteins except that of a 68,000 dalton protein whose amount is increased in the DMSO treated cells (figure 1C). This protein may be albumin since DMSO is known to increase the content of albumin in mouse hepatoma cells[5].

When the *in vivo* phosphorylation of the DBPs of the Yoshida ascites cells was studied, two DBPs of molecular weights 34,000 and 38,000 daltons and a 115,000 dalton DBP showed higher rates of phosphorylation (figure 2) (lanes A and B). S-30 of DMSO treated cells showed the presence of a major phosphoprotein band of molecular weight 34,000 daltons (figure 2B). The extent of stimulation of phosphorylation of this protein by DMSO was determined from the densitometric scanning of lanes A and B of figure 2. The scanning profile depicted in figure 3 shows a six-fold stimulation by DMSO of phosphorylation of the 34 KD DBP. Under the same conditions, DMSO stimulated the phosphorylation of the 38 KD DBP by two-fold and inhibited the phosphorylation of the 115 KD DBP.

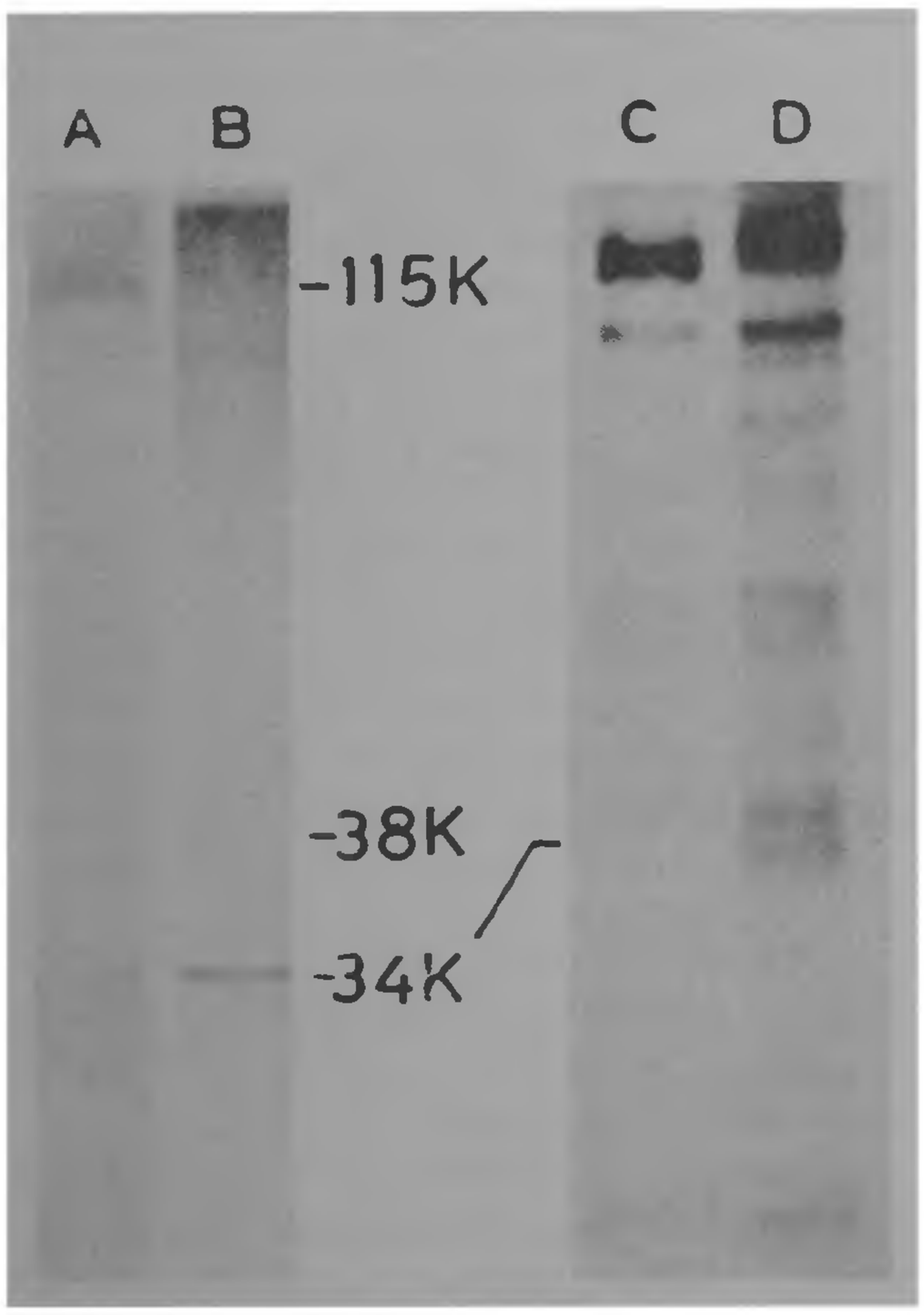


**Figure 2.** Autoradiographic pattern of phosphorylated DNA-binding proteins of Yoshida ascites tumor cells. **A.** phosphorylated DBPs of control cells; **B.** phosphorylated DBPs of DMSO treated cells. Lanes **C** and **D** contain the total phosphorylated proteins of control (**C**) and DMSO treated cells. The proteins were electrophoresed in 10% polyacrylamide gels containing SDS.

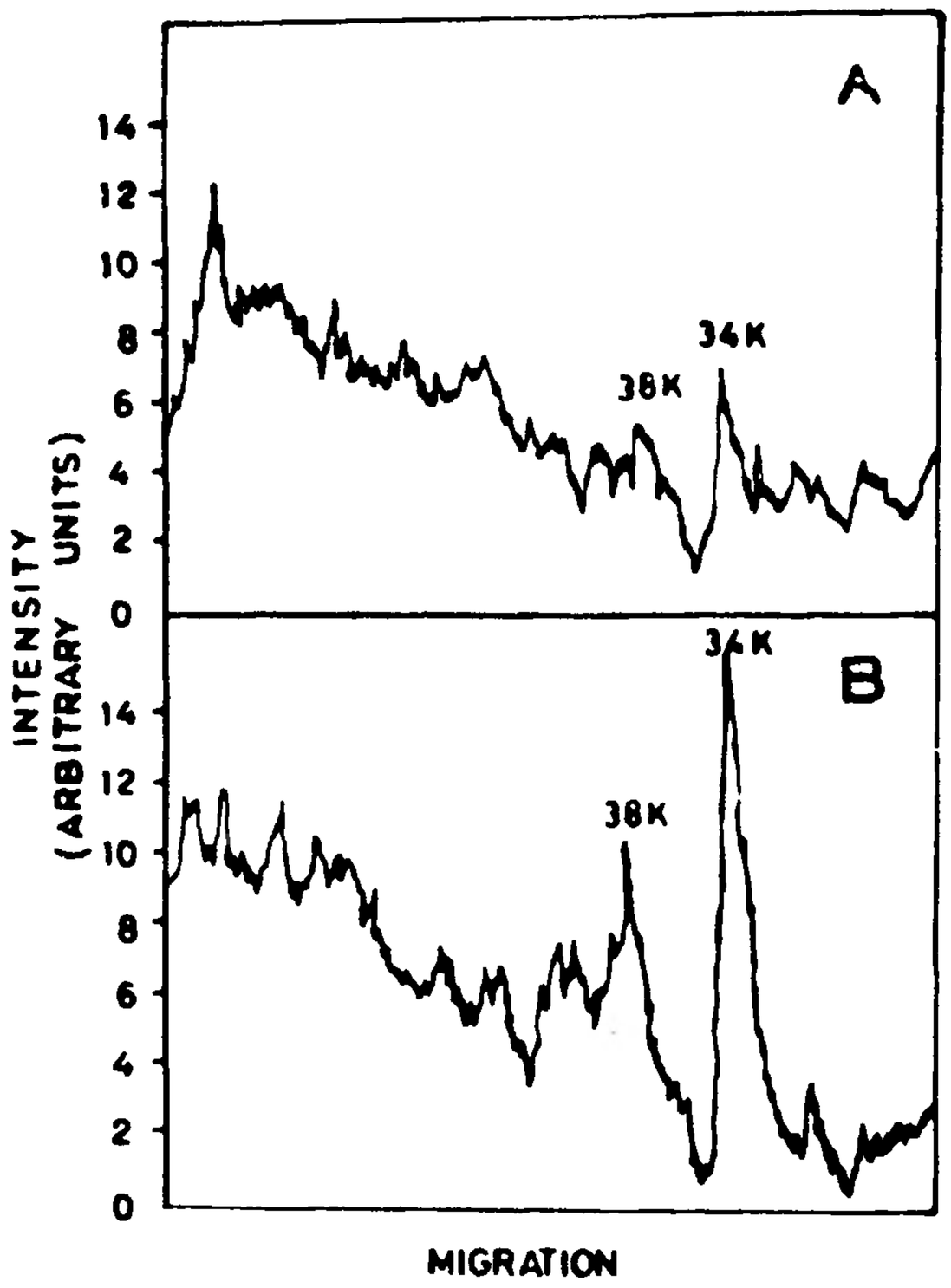


**Figure 3.** Stimulation of phosphorylation of 34 KD and 38 KD DBPs by DMSO. **A** and **B**, respectively depict the densitometric scanning profiles of phosphorylated DBPs of control and DMSO treated cells. These profiles were obtained by scanning the autoradiogram of lanes **A** and **B** of figure 2.

The polar solvent dimethyl sulphoxide exerts a number of unexplained effects on cultured cells. The solvent induces differentiation and haemoglobin production in Friend erythroleukemic cells[6]. Recent studies indicate selective stimulation of tyrosine residue phosphorylation of a growth factor receptor by DMSO[3]. Since DMSO selectively stimulates tyrosine residue phosphorylation[3] the DMSO mediated increase in phosphorylation of the 34 KD DBP may be due to tyrosine specific phosphorylation of this protein. Although these studies have to await confirmation by phosphoamino acid analysis, the observation of specific stimulation by DMSO of phosphorylation of the 34 KD DBP which was shown to possess endonuclease activity[2] is interesting in the light of the report of

inactivation of topoisomerases by tyrosine protein kinases[4].

## ACKNOWLEDGEMENTS

This study was supported by a grant from the Department of Science and Technology, Government of India.

18 November 1985

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# ANNOUNCEMENTS

## INTERNATIONAL SEMINAR ON STRUCTURE AND FUNCTION OF ENZYMES

The International Seminar on Structure and Function of Enzymes will be held on *October 22–25, 1986* at the Department of Biochemistry, Faculty of Science, Banaras Hindu University, Varanasi, India. Papers are invited on this broad theme.

The papers should reach Varanasi *before May 31, 1986*. Authors from outside India may kindly allow sufficient time for the overseas mail which may sometimes take 2–3 weeks.

Details can be had from: Prof. O. P. Malhotra, Department of Biochemistry, Faculty of Science, Banaras Hindu University, Varanasi 221 005, India.

## DIAGNOSTIC MAINTENANCE WORKSHOP '86, IIT, DELHI

Industrial Tribology, Machine Dynamics and Maintenance Engineering Centre (ITMMEC) at Indian Institute of Technology, Delhi will hold a workshop on Diagnostic Maintenance from *November 26–28, 1986*.

The basic objective of the present workshop is to bring together this need of the Industry and state of the art knowledge and expertise available from both within and outside the country. Each of the session will deal with a specified topic in the form of lectures, case-studies and discussions relevant to industries. Experts from Norway, Sweden, U.K., U.S.A. and other countries are likely to participate.

For details please contact: Dr. V. A. Eshwar, Co-ordinator, Diagnostic Maintenance Workshop '86, ITMMEC, Indian Institute of Technology, Hauz Khas, New Delhi 110016.