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Fractionation of RNA polymerase II transcription factors from Hela cell nuclear extracts by affinity chromatography on "DNA-like" phosphorylated polystyrene

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Abstract

It was previously shown that phosphorylated cross-linked polystyrene derivatives specifically interacted with anti-DNA antibodies and anti-phospholipid antibodies present in the sera of systemic lupus erythematosus patients. These resins are potential candidates as stationary phases in affinity chromatography. We wondered whether these biospecific resins might allow the fractionation of DNA binding proteins such as RNA polymerase II transcription factors from HeLa cell nuclear extracts. Indeed, these proteins play a major role in gene regulation in mammalian cells and their purification still requires numerous steps. To study the biospecificity of DNA-like phosphorylated polystyrene derivatives, ethanolamine sulfamide crosslinked polystyrene derivatives were phosphorylated at various rates and HeLa cell nuclear extracts were adsorbed on these resins. Adsorbed proteins were eluted with increasing concentrations of aqueous potassium chloride. Collected fractions were characterized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and the biological activities of the eluted transcription factors were tested by in vitro transcription assay. Results showed that the elution of transcription factors depended on the substitution rate in phosphoester groups of the resins. It appears that specific interactions were developed between the polymers and the transcription factors. Moreover, the eluted transcription factors kept their biological activity. These results lead us to propose the purification of RNA polymerase II transcription factors using the phosphorylated polystyrene resins as stationary phases. © 1997 Elsevier Science B.V.

Keywords: Polystyrene; RNA polymerase II

1. Introduction

Transcription of protein-encoding genes in higher eukaryotic cells is carried out by a multienzymatic complex, including RNA polymerase II (RNA pol.II) and several transcription factors divided into two classes. The first class, termed general transcription factors, acts through the core promoter elements (TATA-box and initiator element) and is required for basal level of specific transcription initiation at all class II promoters [1,2]. At present, six human general transcription factors, TFIIA, -B, -D, -E, -F and -H, are relatively well characterized and are sufficient in addition to RNA pol.II for specific transcription initiation from minimal promoter-containing elements. The second class of transcription factors are DNA-binding proteins that recognize specific promoter or enhancer elements through

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which they regulate gene or tissue-specific transcription [3]. One of these factors is the ubiquitous upstream stimulatory factor (USF) which stimulates transcription ten-fold when it binds to its specific sequence [4–6]. The mechanisms of assembly of RNA polymerase II transcription factors (RNA pol.II-TF) on the promoter, and the initiation of transcription begin to be understood [7–9]. Binding of TFIID complex to the TATA box via the TATA-binding protein (TBP), appears to be the first step in the formation of the transcription complex, providing a recognition site for the assembly of the other general transcription factors and RNA pol.II. This step seems to be facilitated by the interaction of TFIIA with TBP. Then, TFIIB, RNA pol.II brought by TFIIF, TFIIE and TFIIH bind the TFIIA-TBP complex. Fig. 1 represents the initiation transcription complex of RNA pol.II on the major late promoter of Adenovirus 2 (Ad2-MLP), i.e., the model considered in the present study.

However, not all these transcription factors are available despite numerous efforts and the development of various chromatographic processes. Consequently, the mechanism of transcription factor assembly remains controversial. One limitation is that research groups use different purification procedures, with low yields resulting from numerous chromatographic steps [10]. The procedure can be improved and simplified, using affinity chromatography. For some transcription factors, one way is to use known DNA sequences which bind these transcription factors. Another approach is to find an affinity by the variation of the distribution of interaction sites grafted on a stationary phase. Indeed, random phosphorylated polystyrene derivatives have been demonstrated to exhibit DNA-like properties [11,12] by their specific interactions with anti-DNA antibodies from systemic lupus erythematosus. We previously reported that a 72% phosphorylated polystyrene derivative interacts specifically with RNA pol.II transcription factors [13]. In the present work, we report the fractionation of RNA pol.II transcription factors on ten different phosphorylated polystyrene polymers. In order to test whether DNA-like polymers could be used as stationary phases in affinity chromatography, the eluted proteins were assayed by electrophoresis and by in vitro transcription for their biological activity.

2. Experimental

2.1. Materials

Crosslinked polystyrene resins, BioBeads SX2 (Bio-Rad Labs., Richmond, CA, USA), were washed successively with 1 *M* NaOH and 1 *M* HCl. The beads were subsequently washed with distilled water and dried at 50°C under vacuum.

All solvents, including dichloromethane, trimethylphosphate and dimethylsulphoxide (DMSO) were supplied by Carlo Erba (Rueil-Malmaison, France). Monochlorosulfonic acid, ethanolamine, phosphorus oxychloride were purchased from Aldrich (St. Quentin Fallavier, France). Tetrabutylammonium perchlorate (TBAP) was provided by Sigma (St. Quentin Fallavier, France). Elemental analysis were performed by the Service Central d'Analyses CNRS (Vernaison, France).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis were performed with a PhastSystem device using Phast Gel from Pharmacia (Paris, France). Phosphorylase B, bovine albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor and lactalbumin were supplied by Sigma.

α^{32}P-UTP and autoradiography films (Hyperfilm

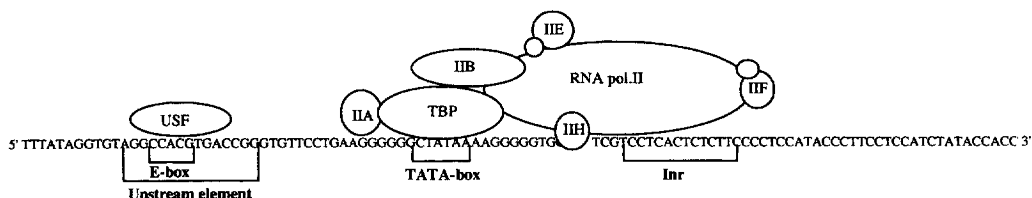

Fig. 1. Diagram of the iniation complex of RNA polymerase II on the adenovirus 2 major late promoter. USF: upstream stimulatory factor; IIA: TFIIA; IIB: TFIIB; IIE: TFIIE; IIF: TFIIF; IIH: TFIIH; TBP: TAT-binding protein (adapted from Refs. [2,8]).

MP) were provided by Amersham (Les Ulis, France). All molecular biology reagents, ATP, CTP, UTP, 3-O-methyl-GTP, RNase inhibitor (RNasin) and Ban I were purchased from Pharmacia, except proteinase K provided by Boehringer Mannheim (Meylan, France).

2.2. Phosphorylated polystyrene derivatives

Crosslinked polystyrene resins were derivatized with –OH end groups (PS-OH) and then phosphorylated by phosphorus oxychloride, as previously described [11,14]. Briefly, 10 g of crosslinked polystyrene in 300 ml dichloromethane were first chlorosulfonated using 70 ml of monochlorosulfonic acid, and then reacted with 100 ml ethanolamine in order to reach the hydroxylated PS–OH. These two reactions were performed in order to obtain almost total substitution of styrene with hydroxyl groups. Phosphorylation at various rates was then achieved by varying the amount of phosphorus oxychloride in the reaction. The resulting polymers were characterized by acidimetric titration and elemental analysis as described before [14].

Polymers were washed with Tris buffer containing 2 *M* of potassium chloride, and water to remove synthesis residues. Resins were extensively rinsed with the adsorption buffer (50 m*M* KCl, 50 m*M* Tris, 0.1 m*M* EDTA, 10% glycerol) equilibrated at pH 7.9. The concentration of the resin was adjusted to 200 mg/ml. Resins were passivated by bovine serum albumin (BSA) as followed: 50 mg of phophorylated resins were incubated for 30 min with 0.4 g/l BSA solution. The passivated resins were rinsed five times with adsorption buffer and were stored at 4°C.

2.3. Transcription factors

RNA pol.II transcription factors contained in HeLa cell nuclear extracts (HNE) were prepared from HeLa cell nuclei (gift of Prof. A. Miller, Mons, Belgium) according to Gorski et al. [15]. Experiments were performed at 4°C, and before use, 1 m*M* dithiothreitol and 0.5 m*M* PMSF as protease inhibitors were added.

Two different pools of HNE were used. One was a gift of Prof. A. Miller (HNE_1). The other one (HNE_2) was prepared as follow: HeLa cell nuclei were resuspended in solution A (0.14 *M* NaCl, 20 m*M* Hepes pH 7.9, 25% glycerol, 1.5 m*M* $MgCl_2$, 0.5 m*M* EDTA). Solution B (0.7 *M* NaCl, 20 m*M* Hepes pH 7.9, 25% glycerol, 1.5 m*M* $MgCl_2$, 0.25 m*M* EDTA) was added in order to obtain an NaCl final concentration of 0.37 *M*. This nuclei suspension was incubated for 30 min under agitation and centrifuged for 30 min at 112 080 *g*. The supernatant was precipitated with 0.3 g/ml ammonium sulfate for 30 min under gentle agitation, and centrifuged for 20 min at 112 000 *g*. The pellet was incubated for 10 min in dialysis solution (20 m*M* Hepes pH 7.9, 60 m*M* KCl, 20% glycerol, 0.25 m*M* EDTA) containing protease inhibitors mixture (0.5 m*M* PMSF, 5 μg/ml aprotinin, 5 μg/ml pepstatin, 5 μg/ml leupeptin) and dialysed overnight. HeLa cell nuclear extracts were stored in liquid nitrogen.

Protein concentration was determined by the Bradford protein assay [16] using BSA as standard.

2.4. Adsorption and elution procedures

All adsorption and elution experiments were performed at 4°C and repeated twice in order to verify for the reproducibility. To reduce the contact-time between proteins and polymer, and between proteins and high-ionic strength solutions, two adsorption and elution runs were performed in parallel experiments. In each run, 300 μg of HNE were incubated with 50 mg of the phosphorylated resin, under rotational stirring (12 rpm) for 15 min. The resin was washed with the adsorption buffer and 10 mn batch-eluted with 0.5 ml of KCl solution of different concentrations. In the first run, the step gradient was 0.2, 0.6, 1, 1.4 and 1.8 *M* KCl, and for the other run, the step gradient was 0.4, 0.8, 1.2, 1.6 and 2 *M* KCl. The collected fractions were dialysed against the adsorption buffer, and concentrated using Centricon 10 (10 000 cutoff, Amicon). The fractions were stored at −20°C.

2.5. Protein characterization by electrophoresis

Elution fractions were analyzed by SDS–PAGE using 12.5% homogeneous Phast Gel (Pharmacia) following electrophoresis on a PhastSystem device (Pharmacia). The reference protein mixtures were

made up for both molecular mass and concentration standard: phosphorylase B (94 000, 3 μg/ml), bovine albumin (67 000, 2.5 μg/ml), ovalbumin (43 000, 2 μg/ml), carbonic anhydrase (30 000, 1.5 μg/ml), trypsin inhibitor (20 100, 1 μg/ml) and α-lactalbumin (14 400, 0.5 μg/ml). Gels were stained with silver nitrate. The position and intensity of the bands were quantitatively characterized as previously described [13].

2.6. *In vitro transcription assay*

In vitro transcription assays were performed using the plasmid pML(C_2AT) which contains the Adenovirus type 2 major late promoter linked to a "G-free" cassette (gift of Prof. M. Yaniv, Institut Pasteur, Paris, France). Assays were carried out in a final volume of 20 μl containing 100 ng of pML(C_2AT) with either 10 μl of HNE (30 μg) or elution fractions, and 10 μl of 15 m*M* HEPES (pH 7.9), 25 m*M* KCl, 6 m*M* $MgCl_2$, 0.6 m*M* ATP and CTP, 5 m*M* UTP, 0.1 m*M* 3-O-methyl-GTP, 15 μCi ^{32}P-labeled UTP (10 mCi/ml), 30 units of RNasin [17]. Reaction mixtures were incubated at 30°C for 1 h. Transcription reactions were stopped by the addition of 280 μl of a solution containing 0.25 m*M* NaCl, 1% SDS, 20 m*M* Tris (pH 7.5), 5 m*M* EDTA, followed by a proteinase K digestion (40 μg) for 30 min at 30°C. After phenol–chloroform extractions and ethanol precipitation, the products of the in vitro transcription were characterized by electrophoresis on a 6% polyacrylamide-urea (7 *M*) sequencing gel (17.5×14 cm) followed by a 2 day exposure with a Hyperfilm MP at −80°C.

Size standard composed of DNA fragments was obtained from the digestion of pBR322 plasmid (gift of Prof. M. Yaniv) by the restriction enzyme Ban I.

2.7. *Quantitative analysis*

SDS–PAGE and in vitro transcription autoradiograms of the eluted fractions were analyzed using the image analyzer BIOCOM 200 which allows to determine the molecular mass and the relative concentration of each protein. Detection was carried out by comparison of band intensities (total intensities of the bands from which the background was substracted), with those of the reference.

3. Results and discussion

3.1. *Characterization of phosphorylated polystyrene derivatives*

The synthesis of polystyrene derivatives phosphorylated at various rates was achieved by varying the amount of phosphorus oxychloride in the reaction. Characterization of the resulting polymers was performed by elemental analysis and acidimetric titration. Results are presented in Table 1. We have previously demonstrated [14] that phosphorylation of

Table 1
Chemical composition of phosphorylated polystyrene derivatives

Resins	PS	PS-SO_3^-	PS-OH	PS-P	PME	PDE
PS-P6	6.5	4.5	76.5	6	0.3	12
PS-P11	10.5	2.5	65.5	11	0.6	21
PS-P23	10	4	41.5	23	2	42
PS-P31	15	0	31	31	8	46
PS-P37	7	0	32	37	12.5	49
PS-P41	13.5	3	18	41	17	48
PS-P46	10	3	16.5	46	22	48
PS-P52	11.5	10	0	52	28	48
PS-P57	3.5	0	13	57	31	52
PS-P72	0	1	0	72	45	54

PS: Non substituted styrene unit; PS-SO_3^-: styrene unit with sulfonate groups; PS-OH: styrene unit with hydroxylated groups; PS-P: total phosphate groups grafted on the polymer; PME: styrene unit with phosphomonoester groups; PDE: styrene unit with phosphodiester groups. Values are % of subsituted styrene units (PS+PS-SO_3^-+PS-OH+PME+PDE=100%), except PS-P which is the % of substituted phosphate groups (PS-P=PME+0.5·PDE).

ethanolamine sulfamide cross-linked polystyrene led to the formation of phosphodiesters (PDE) and phosphomonoesters (PME) groups, in amounts varying with the total phosphorylation rate of the polymer. In all cases, more than 85% of styrene units and 90% of sulfonate units were substituted indicating almost total conversion. Phosphorylation range from 6% to 72% was obtained with polymers displaying the same content in PDE and different contents in PME (PS-P37, PS-P41, PS-P46, PS-P52), and polymers displaying different contents in PDE and PME (PS-P6, PS-P11, PS-P23, PS-P31, PS-P57, PS-P72). These characteristics allowed us to study the influence of the phosphorylation rate on RNA pol.II-TF adsorption.

3.2. Adsorption and elution of RNA pol.II transcription factors

Two different preparations of HNE containing all the transcription factors were incubated with the phosphorylated resins. Adsorbed proteins were eluted from resins by KCl gradient and the protein content was assessed by quantitative image analysis of silver stained SDS–PAGE of each elution fraction. Each polymer was tested twice with the same or different batches of HNE.

Seven transcription factors, namely TFIIA, -B, -E, -F, -H, TBP (TATA-binding protein) and USF, in addition to RNA pol.II [1,18] were studied. Identification of these proteins was performed as described [13,19–26]. As an example, results obtained from PS-P41 resin are presented in Table 2. All RNA pol.II-TF in addition to RNA pol.II were found in fractions eluted up to 0.6 *M* KCl. In other fractions eluted at higher KCl concentrations, some transcription factors were indicated as missing according to SDS–PAGE analysis. Notably, TFIIH was not detected in fractions eluted at 0.8 *M*, 1 *M* and 1.2 *M* KCl; TFIIA was absent in fractions eluted at 1 *M* and 1.2 *M* KCl. In 1.2 *M* KCl, USF was the only transcription factor detected. These results indicated that the concentration of each "missing" transcription factor was below the sensitivity of the silver nitrate staining, which is estimated at 1 ng/μl. Furthermore, SDS–PAGE quantitative analysis gave some information about the purification level of RNA pol.II-TF on PS-P41. As an example, in fraction eluted at 0.6 *M* KCl the calculated amount for TFIIB, TBP, TFIIE, TFIIF, TFIIH and USF are respectively 31.6 ng, 89.2 ng, 123.4 ng, 104 ng, 87.8 ng and 52 ng, and below the sensitivity of the method for TFIIA and RNA pol.II. In 1.2 *M* KCl, 44.5 ng of a protein displaying the same electrophoretic characteristics than USF and none of the other transcription factors were recovered from 300 μg of HeLa cell nuclear extract. As comparison, the purification of pure USF was already described by Moncollin et al. [27] and they recovered 2.5 ng of USF from 1.16 g of nuclear extract on DNA-affinity chromatography.

Moreover, the KCl concentration required for maximal elution of a given transcription factor is related to the binding free energy of the phosphorylated polystyrene derivative [13]. The binding free energy correlated to the affinity constant for a protein [28]; the higher the elution ionic strength, the higher the affinity. Results of maximal desorption indicated that transcription factors were eluted from PS-P41 with various ionic strengthssuggesting that they displayed different affinities for this polymer (Table 3). USF showed the highest KCl concen-

Table 2
Protein content of elution fractions obtained after adsorption of HeLa cell nuclear extracts (HNE_2) on PS-P41

[KCl] (*M*)									
Run 1	Run 2	TFIIA	TFIIB	TBP	TFIIE	TFIIF	TFIIH	USF	RNA pol.II
0.2		+	+	+	+	+	+	+	+
	0.4	+	+	+	+	+	+	+	+
0.6		+	+	+	+	+	+	+	+
	0.8	+	+	+	+	+	+	+	+
1		−	+	+	+	+	−	+	−
	1.2	−	−	−	−	−	−	+	−

The presence (+) or the absence (−) of each transcription factor was determined by SDS–PAGE analysis.

Table 3
Maximal ionic strength required for the elution of RNA polymerase II transcription factors and RNA polymerase II (pol.II) from PS-P41

	TFIIA	TFIIB	TBP	TFIIE	TFIIF	TFIIH	USF	pol.II
[KCl] (*M*)	0.5±0.1	0.7±0.1	0.7±0.1	0.7±0.1	0.7±0.1	0.5±0.1	0.9±0.1	0.5±0.1

Values are mean±S.D of at least two separate determinations.

tration for maximal elution at 0.9±0.1 *M* indicating that USF displayed the highest affinity for PS-P41. TFIIB, TBP, TFIIE and TFIIF displayed the same KCl concentration for maximal elution at 0.7±0.1 *M*, indicating that PS-P41 developed less interactions with these transcription factors than with USF. TFIIA and RNA pol.II required a KCl concentration of 0.5±0.1 *M* to obtain maximal elution. TFIIH showed the lowest KCl concentration for maximal elution at 0.3±0.1 *M*. Therefore, the affinity of the different transcription factors for PS-P41 increased in the following order: TFIIH<TFIIB=TBP=TFIIE=TFIIF<USF.

Ten polymers were studied and analyzed in the same way. Transcription factor content of fractions eluted at each KCl concentration was obtained considering only one adsorption and elution run of HNE on phosphorylated polymers (Table 4). For example, fraction eluted with 1.2 *M* KCl from PS-P41 (run 2) contained proteins eluted between 0.8 *M* and 1.2 *M* KCl (Table 2). Furthermore, fraction eluted with 1 *M* KCl (run 1) contained proteins eluted between 0.6 *M* and 1 *M* KCl (Table 2). Because no transcription factors were detected in fractions eluted with KCl concentrations higher than 1.2 *M* (cf. Table 2), we deduced that with one adsorption and elution run of HNE on PS-P41, fraction eluted with 1 *M* KCl contained only USF and fraction eluted with 0.8 *M* KCl contained TFIIB, TBP, TFIIE, TFIIF and USF (Table 4). The same analysis was performed for each elution fraction obtained from all polymers. Results indicated that fractions eluted at 0.2 *M* KCl all contained RNA pol.II-TF. It appeared that in some elution fractions all transcription factors in addition to RNA pol.II were present whereas in others one or more of the transcription factors were missing. Moreover, some RNA pol.II-TF were isolated after a single adsorption step on phosphorylated resins. Notably, TBP was isolated from PS-P6 and PS-P72 in fractions 1 *M* and 1.2 *M* KCl (15.9 ng of TBP) respectively, USF was isolated from PS-11 (fraction 0.6 *M* KCl), PS-P31 (fraction 0.6 *M* KCl: 1 ng of USF), PS-P37 (fraction 0.6 *M* KCl: 11.2 ng of USF), PS-P41 (fraction 1 *M* KCl: 44.5 ng of USF), PS-P46 (fraction 1.4 *M* KCl: 7 ng of USF), PS-P52 (fraction 1.2 *M* KCl: 40.6 ng of USF) and PS-P57 (fraction 1.2 *M* KCl: 7.4 ng of USF), and TFIIE was isolated from PS-P11 in fractions 1 *M* and 1.2 *M* KCl.

Moreover, data presented in Table 4 indicated that phosphorylated polystyrene derivatives developed different interactions with RNA pol.II-TF depending on the phosphorylation rate. PS-P31 and PS-P37 developed low and reversible interactions with transcription factors, the highest KCl concentration for maximal elution being 0.6 *M* for USF, 0.4 *M* for TFIIB and 0.2 *M* for the other transcription factors. These results suggested that 31% and 37% of phosphorylation rate did not lead to the creation of sites which could interact specifically with RNA pol.II-TF. On the contrary, PS-P6, PS-P11, PS-P23, PS-P41, PS-P46, PS-P52, PS-P57 and PS-P72 developed low and high, and reversible, interactions with RNA pol.II-TF. Indeed, transcription factors were eluted from these resins with low ionic strengths (below 0.8 *M* KCl) indicating that proteins were adsorbed on polymer sites with a low affinity. Furthermore, RNA pol.II-TF were also eluted with high ionic strengths (above 0.8 *M* KCl) indicating that these proteins were adsorbed on other polymer sites with a high affinity. For example, all transcription factors were eluted from PS-P23 with 0.2 *M* KCl suggesting interactions with low affinity sites on the resin, but the highest KCl concentrations for maximal elution on PS-P23 of TFIIA and TFIIF was 1 *M*, of TFIIE and USF was 1.2 *M*, of TFIIB and TBP was 1.6 *M* suggesting interactions with high affinity sites.

However, it was important to test the biological activity of the eluted transcription factors and to

Table 4
RNA polymerase II transcription factor content of fractions obtained after KCl elution of HeLa cell nuclear extracts adsorbed on phosphorylated polystyrene derivatives

[KCl] (*M*)	PS-P6	PS-P11	PS-P23	PS-P31	PS-P37	PS-P41	PS-P46	PS-P52	PS-P57	PS-P72
0.4	TFIIA	TFIIA	TFIIA			TFIIA	TFIIA	TFIIA	TFIIA	TFIIA
	TFIIB	TFIIB	TFIIB	TFIIB	TFIIB	TFIIB	TFIIB	TFIIB	TFIIB	TFIIB
	TBP	TBP	TBP			TBP	TBP	TBP	TBP	TBP
	TFIIE	TFIIE				TFIIE	TFIIE	TFIIE	TFIIE	TFIIE
	TFIIF	TFIIF				TFIIF	TFIIF	TFIIF	TFIIF	TFIIF
	TFIIH	TFIIH				TFIIH	TFIIH	TFIIH	TFIIH	TFIIH
	USF	USF	USF	USF	USF	USF	USF	USF	USF	USF
	pol.II	pol.II	pol.II			pol.II	pol.II	pol.II	pol.II	pol.II
0.6	TFIIA		TFIIA			TFIIA	TFIIA	TFIIA	TFIIA	TFIIA
	TFIIB		TFIIB			TFIIB	TFIIB	TFIIB	TFIIB	TFIIB
			TBP			TBP	TBP	TBP	TBP	TBP
	TFIIE					TFIIE	TFIIE	TFIIE	TFIIE	TFIIE
	TFIIF					TFIIF	TFIIF	TFIIF	TFIIF	TFIIF
	TFIIH					TFIIH	TFIIH	TFIIH	TFIIH	TFIIH
	USF	USF	USF	USF	USF	USF	USF	USF	USF	USF
	pol.II		pol.II			pol.II	pol.II	pol.II	pol.II	pol.II
0.8	TFIIA	TFIIA	TFIIA				TFIIA	TFIIA	TFIIA	TFIIA
	TFIIB	TFIIB	TFIIB			TFIIB	TFIIB	TFIIB	TFIIB	
		TBP				TBP	TBP	TBP	TBP	
	TFIIE					TFIIE	TFIIE	TFIIE	TFIIE	TFIIE
	TFIIF	TFIIF	TFIIF			TFIIF		TFIIF	TFIIF	TFIIF
	TFIIH	TFIIH					TFIIH	TFIIH	TFIIH	TFIIH
	USF		USF			USF	USF	USF	USF	USF
	pol.II	pol.II	pol.II				pol.II	pol.II	pol.II	pol.II
1			TFIIA				TFIIA			
			TFIIB				TFIIB	TFIIB		TFIIB
	TBP							TBP	TBP	TBP
		TFIIE					TFIIE	TFIIE		
			TFIIF					TFIIF		
							TFIIH	TFIIH		
			USF			USF	USF	USF	USF	
			pol.II				pol.II	pol.II		
1.2										
							TBP			TBP
		TFIIE	TFIIE							
							TFIIF			
			USF				USF	USF	USF	
							pol.II			
1.4		TFIIA								
		TFIIB								
		TBP								
		TFIIF								
		TFIIH								
							USF			
		pol.II								
1.6										
			TFIIB							
			TBP							

confirm that the eluted fractions contained the RNA pol.II-TF.

3.3. Transcriptional activity of the eluted transcription factors

Biological activities of the eluted transcription factors obtained from two different pools of HNE were tested using an in vitro transcription assay reconstituted with each elution fraction and pML(C_2AT), a plasmid described by Sawadogo and Roeder [17] containing the adenovirus 2 major late promoter from −400 to +10 bp linked to a 400 bp "G-free" cassette. Transcription reactions were performed without GTP and with 3-O-methyl-GTP to prevent random transcription initiation. Separation of transcription product was carried out on a 6% sequencing gel in comparison with size standard DNA fragments. According to published data [1,2], all the general transcription factors and RNA pol.II were required to initiate specific transcription from the Ad2-MLP.

A representative example of in vitro transcription autoradiography of elution fractions obtained from adsorption of HNE_1 on PS-P46 is presented in Fig. 2. A transcription signal was obtained from elution fractions at 0.2 *M*, 0.4 *M*, 0.6 *M*, 0.8 *M* and 1.2 *M* KCl, indicating that RNA pol.II-TF were present and biologically active. For higher KCl concentrations and for 1 *M* KCl, no transcription was observed. The absence of transcription demonstrates that at least one of the RNA pol.II-TF was missing as suggested by SDS–PAGE analysis (Table 4). In vitro transcription experiments performed with the starting HNE_1 and with elution fractions, containing all RNA pol.II-TF, gave similar results.

Furthermore, the transcriptional activity was quantified using an image analyzer to determine specific transcription activity of each elution fraction obtained from the two adsorption/elution runs on all polymers. Results are reported in Table 5. For each resin it appeared that the relative transcriptional activities of the flow-through (FT) eluted at 0.05 *M* KCl were higher than those of the starting HNE. Adsorption of HNE on phosphorylated resins led to an increase of the apparent specific transcriptional activity from 2-fold (PS-P52) to 34-fold (PS-P6) for

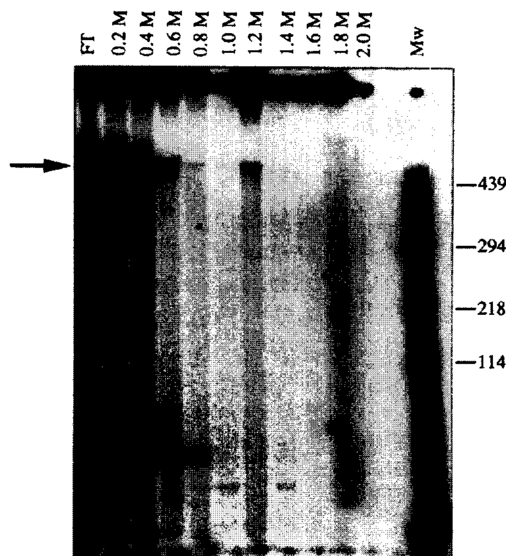

Fig. 2. In vitro transcription autoradiogram of elution fractions (0.2 to 2 *M* KCl) from PS-P46. Specific transcription product was observed (arrow) at about 515 bp. FT: flow-through fraction at 0.05 *M* KCl; M_w: DNA molecular mass standard in bp.

the FT fraction obtained from HNE_1 and from 6-fold (PS-P23) to 540-fold (PS-P72) for the FT fraction obtained from HNE_2. These results suggested that the starting HNE contained components which disturbed or modulated transcriptional mechanism.

In addition to the specific transcript (515 pb), few smaller transcripts were produced from HNE_1-elution fractions. The amount of these small transcripts decreased as KCl elution concentration increased up to 0.6 *M* KCl where only the specific transcript was observed (Fig. 2). These data suggested that HNE_1 contained contaminant proteins disturbing specific transcription which weakly interacted with phosphorylated polystyrene derivatives. Adsorption of HNE_1 on phosphorylated resins allowed to separate transcription factors from these contaminant components. Moreover, the small obtained transcripts suggested that contaminant components might be RNases which damaged the specific transcript. On the contrary, in vitro transcription performed with the initial HNE_2 did not produce the expected specific transcript but a smaller RNA at about 70 pb whereas elution fractions produced only the specific

Table 5
Specific in vitro transcriptional activities of elution fractions obtained from adsorption/desorption of HeLa cell nuclear extracts on phosphorylated polystyrene derivatives

[KCl] (*M*)	PS-P6	PS-P11	PS-P23	PS-P31	PS-P37	PS-P41	PS-P46	PS-P52	PS-P57	PS-P72
HNE	1.43	0.43	0.1	0.13	0.1	0.1	2.3	1.73	0.77	0.07
FT	48.3	10.9	0.57	3.1	8.15	0.74	72.7	4.2	10	37.8
0.2	33.7	34	0.75	1.67	20	20	50.3	15.3	51	22.6
0.4	34	40	0.18	1.87	0	14.7	47.5	14.2	9.75	19.4
0.6	n.d.	108	0	0	0	48.3	13.2	36.5	9.4	20
0.8	0	0	0	0	0	n.d.	0[1]	205	13.7	n.d.
1	n.d.	n.d.	0	0	0	0	0	n.d.	n.d.	25.4
1.2	0	0	0	0	0	0	n.d.	n.d.	0	0
1.4	0	n.d.	0	0	0	0	0	0	0	0

Specific transcriptional activity of each elution fraction was calculated as the ratio of the relative specific transcript intensity obtained by densitometry analysis to the total protein concentration determined by the Bradford protein assay.
[1] Fraction eluted with 0.8 *M* KCl from PS-P46 did not produced the specific transcript (515 pb) but a smaller RNA about 60 pb.
HNE: Starting HeLa cell nuclear extract.
FT: Flow-through fraction eluted at 0.05 *M* KCl.
n.d.: Not determined.

transcript at 515 pb. HNE_2 probably contained additional contaminant components that are different from those of HNE_1. Adsorption of HNE_2 on phosphorylated polystyrene derivatives separated the transcription factors from these contaminant components which might be elongation inhibitors.

Adsorption/elution from HNE led to the increase of the apparent specific transcriptional activities from 6-fold (PS-P46: 0.6 *M* KCl) to 251-fold (PS-P11: 0.6 *M* KCl) for elution fractions obtained from HNE_1 and from 2-fold (PS-P23: 0.4 *M* KCl) to 483-fold (PS-P41: 0.6 *M* KCl) for elution fractions obtained from HNE_2 (Table 5). We concluded that adsorption of HNE on phosphorylated polystyrene derivatives allowed the purification of RNA pol.II-TF with a high specific transcriptional activity level. Elution fractions obtained from resins PS-P11, PS-P41, PS-P52, PS-P57 and PS-P72 displayed specific transcriptional activities. The increase of transcriptional activity with KCl elution concentrations suggested that strong and reversible interactions were developed between RNA pol.II-F and these polymers. Results of in vitro transcription also demonstrated that proteins identified as transcription factors by SDS–PAGE analysis were RNA pol.II-TF and that the adsorption/desorption process on phosphorylated polystyrene derivatives did not affect their biological activities.

4. Conclusions

In this study, we reported interactions between RNA pol.II transcription factors and randomly phosphorylated polystyrene derivatives. Adsorption/desorption results showed that the transcription factors displayed different affinities for these polymers. For a given transcription factor, the affinity varied with the phosphorylation rate of the polymer, and for a given polymer, the transcription factors adsorbed with different affinities. These adsorptions were reversible and the RNA pol.II-TF kept their biological activity as monitored by a specific in vitro transcription assay. Furthermore, our data indicated that the eluted transcription factors were purified from the original nuclear extract. Indeed, the adsorption/elution process of HeLa cell nuclear extracts performed on phosphorylated polystyrene derivatives led to an increase of the transcriptional activities of the eluted fractions by at least 250-fold. Moreover, taking into account the variable elution rates of the transcription factors depending on the resin, phosphorylated polystyrene derivatives might be of interest as stationary phases for the fractionation of RNA pol.II transcription factors by high-performance liquid chromatography [29]. Indeed, TBP, TFIIE and USF were isolated from HeLa cell nuclear extracts, with a good purification rate, after

one chromatographic step on PS-P72, PS-P11 and PS-P41 resins, respectively. As comparison, classical RNA pol.II-TF purification required at least six chromatographic steps [18,28,30]. We assumed that isolation of the other RNA pol.II-TF could be performed using a combination of various polystyrene derivatives. However, fractionation to homogeneity yet remains to be achieved.

Furthermore, we assumed that a phosphorylated polymer might behave as a DNA-like component in its ability to interact with DNA binding proteins. The biospecificity of these phosphorylated polystyrene derivatives was the result of the formation of biospecific sites made up of arrays of PDE and PME [14], the probability of occurrence of which depended on the composition of the polymer. Indeed, it was previously shown that phosphorylated resins specifically interact with anti-DNA antibodies depending on the composition of the polymers [12] and we demonstrated in this study a similar behaviour with RNA polymerase II transcription factors. Work is in progress to determine the relationship between the affinity of RNA pol.II-TF and the composition of the polymers.

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