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Oncoprecipitins: A novel type of natural substances and oncofetal antigens

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Abstract. Immunochemical and physico-chemical properties of two oncofetal antigens - carcinoembryonic antigen (CEA) and pregnancy-specific β_1-glycoprotein (SP-1, PSβG) have been studied. The spatial organization of the protein portion of SP-1 and CEA preparations, as revealed by spectroscopic evidence, has been discussed with respect to their antigenic activity. We found that the SP-1 and CEA protein portion consists mainly of a β-structural type. The antigenic determinants of both preparations were shown to be topographic. It seems, that the orientation of the CEA carbohydrate chains relatively to the protein core plays an important role in the formation of the antigenic sites. SP-1 preparations were found to be capable of splitting p-nitrophenylphosphate, γ-[^{32}P]-ATP and 4-nitrophenyl-5'-TMP. SP-1 was also shown to effectively hydrolyse [^{3}H]-polyuridilic acid and heat-denatured [^{3}H]-DNA of embryos of marine urchin *S.intermedius*. Enzymic hydrolysis proceeds from 3' end of the DNA chain by exonuclease's mechanism. Peculiar glycoproteins, termed as oncoprecipitins have been found in the extracts of various marine invertebrates. The following oncoprecipitins have been isolated and characterized: crustacin from hermit crab *Pagurus prideauxii*, cyprein from cowrie *Cyprea caputserpentis* recognizing CEA and oncoprecipitin A from ascidian *Didemnum ternatum* interacting with SP-1. The interaction pattern of CEA and its oncoprecipitins is similar to that of CEA and its antibody. The possibility of application of CEA oncoprecipitins for serologic tumour diagnosis has been demonstrated. The interaction between oncoprecipitin A and SP-1 differs from that of SP-1 with its antibodies. The effect, produced by oncoprecipitin A on HeLa-M and A-431 tumour cells causes the essential alteration of their cytoskeleton. The incubation of HeLa cells with oncoprecipitin A, followed by SP-1 leads to a recurrent state of their cytoskeleton. The possible role of oncoprecipitins as substances able to monitor the appearance and elimination of neoplastic cells of invertebrates will be discussed.

INTRODUCTION

Human neoplasms produce and release into the circulation a number of substances collectively referred to as tumor markers. One particular group comprising the oncofetal antigens is the most widely used in clinical oncology (Ref. 1) for surveillance of cancer patients, detection of metastases and control of effective therapy. Carcinoembryonic antigen (CEA) is a typical representative of such substances. It is produced both by early embryonic tissue and as a result of gene activation occurring in various carcinomas. Recent immunochemical and biochemical studies have revealed that CEA belong to a family of closely related substances (Ref. 2) which are subdivided into the CEA and pregnancy-specific β_1-glycoprotein (SP-1, PSβG) subgroups (Ref. 3). The SP-1 is known to circulate in large amounts in maternal serum in pregnancy (Ref. 4). All members of the CEA family belongs to the Ig superfamily (Ref. 5). Although CEA and SP-1 have been widely studied, a number of problems, concerning particularly their spatial structure and physiological role, still remain unsolved. The high degree of glycosylation of CEA and SP-1 has made their characterization difficult.

A functional feature of the immunoglobulin gene superfamily proteins is their role in processes of cell-cell and cell-substrate interactions (Ref. 6). More recently it was shown (Ref.7), that members of the CEA family can function as intercellular adhesion molecules. In this connection the search for natural substances, interacting specifically with such molecules, seems to be of great interest especially among those organisms in which neoplasms occur rarely.

Recently, we have found (Ref. 8, 9) that marine invertebrates appear to produce specific substances interacting with CEA and SP-1. These compounds have been named oncoprecipitins.

In this paper, we summarize our experience related to the investigation of immunochemical and physico-chemical properties of SP-1 and CEA preparations. Particular attention has been given to their spatial structure and localization of antigenic determinants. In addition, the data, resulting from the study of three oncoprecipitins are presented.

AN INVESTIGATION OF CEA AND SP-1

Isolation and phsico-chemical characteristics

All procedures of isolation of CEA and SP-1 were carried out at neutral pH values to avoid denaturation of the antigens (Ref. 10, 11). As a result homogeneous CEA and SP-1 preparations have been obtained. Physico-chemical properties (Table 1) and analytical data (Table 2) indicated, that both antigens are sialoglycoproteins with high carbohydrate content, which causes their great intramolecular heterogeneity. Molar extinction of CEA was estimated to be 10% above the calculated one.

TABLE 1.
Physico-chemical properties of CEA and SP-1.

Characteristic	CEA	SP-1
Molecular mass (kDa)	180±10	75±3
Electrophoretic mobility in agar, pH 8,6	β-globulins	β_1-globulins
Molar extinction, E_{278} (cm^2/mol)	103900	47800
Isoelectric point, pI	3.2-4.5	4.0-4.3
Sedimentation constant, $S_{20°,w}$	8±0.25	4.6±0.1
N-terminal amino acid	Lys	-
Protein (%)	47.5	68.6
Neutral sugars (%)	48.0	27.1
Sialic acids (%)	3.1	3.2
Number of S-S linkages	6	4

TABLE 2.
Analytical data of CEA and SP-1.

Amino acid	CEA	SP-1	Amino acid/ Sugar	CEA	SP-1
Asx	87	35	Tyr	22	16
Thr	52	34	Phe	11	8
Ser	71	40	Lys	19	16
Glx	61	36	His	9	6
Pro	50	34	Arg	18	14
Gly	48	47	Trp[2]	10	5
Ala	45	34	Man	78	22
1/2Cys[3]	12	8	Gal	133	37
Val	43	27	Fuc	97	6
Met	0	0	GlcNAc	145	40
Ile	31	27	GalNAc	0	0
Leu	51	35	NeuNAc	20	8

[1] Contents of amino acids and sugars in number of residues on a molecule.
[2] Determined by the second derivative UV-spectrum.
[3] Estimated as carboxymethyl cysteine.

Structure of the carbohydrate chains

The methods of methylation and periodic oxidation were used for the structural studies of CEA and SP-1 sugar chains. Analysis of the methylated sugars obtained on hydrolysis of the permethylated antigens (Ref. 12, 13) demonstrated that the carbohydrate chains of CEA and SP-1 are likely to be N-glycosidically linked oligosaccharides of the N-acetyllactosamine type. The relatively high contents of 2,4-di-O-methyl- and 3,4,6-tri-O-methylmannose as well as the occurrence of 3,6-di-O-methyl-2-N-methyl-acetamido-2-deoxyglucose indicates the presence of a mannotriosido-N,N'-chitobiose "core" in both antigens and a bi-antennary structural pattern (Fig.1a) for most sugar chain in SP-1. The carbohydrate chains of CEA are characterized by a high degree of branching with high content of the terminal fucose and galactose residues. Tri- and tetra-antennary structural patterns as well as a "high mannose" type (Ref. 14) are typical for most sugar chains in CEA (Fig. 1b,c; Ref. 14).

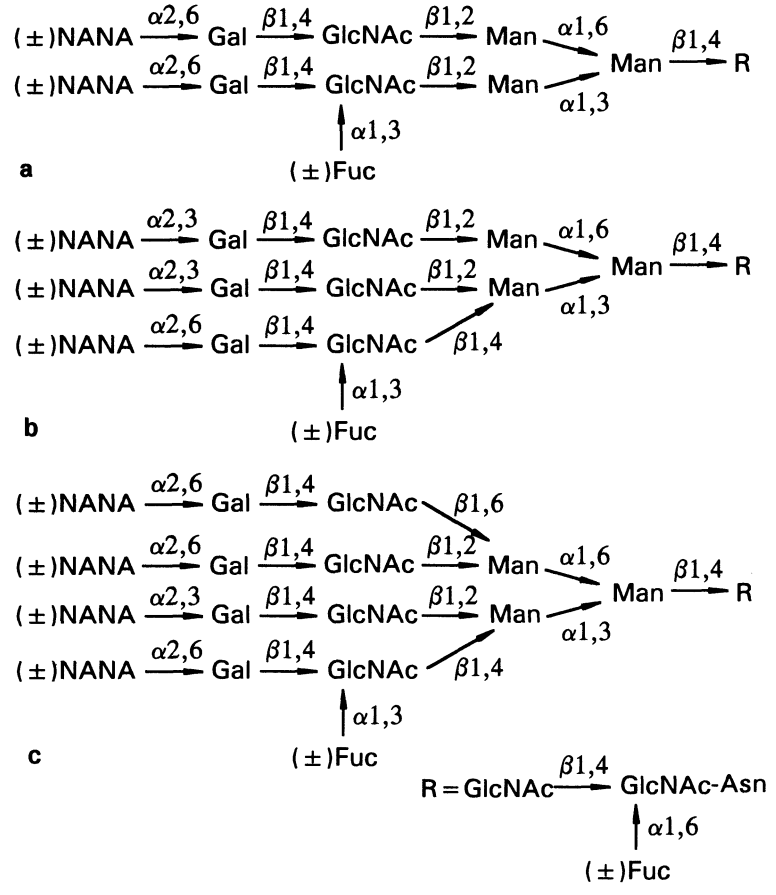

Fig.1 Presumed structures of the carbohydrate chains in CEA and SP-1. **a** Di-antennary chains. **b** Tri-antennary chains. **c** Tetra-antennary chains.

Spatial structure and antigenic activity

Circular dichroism (CD) and differential UV spectroscopy were employed for the evaluation of the spatial organization of the protein portion of CEA and SP-1 (Ref. 15, 16). Immuno enzyme "sandwich" assay with polyclonal rabbit antisera was used to monitor the antigenic activity in both antigens.

Intense positive bands were observed in the aromatic region (230-350 nm) of the CD spectra of CEA and SP-1. These bands are indicative of the occurrence of aromatic amino acids in the interior parts of the CEA and SP-1 molecules. CD spectra of both antigens within the range of absorption of peptide linkages (185-230 nm) are typical of proteins with a high content of β-structures. Calculation of the secondary structural elements (Ref. 17) of CEA and SP-1 protein portions (Table 3) demonstrated that β-structures and β-turns are predominant in both antigens.

Heating CEA and SP-1 solutions from 20 to 80°C led to a conformational transition occurring within the temperature interval 50-60°C for CEA and 45-55°C for SP-1 (Fig. 2, 3). The estimation of the secondary structural elements (Table 3) has shown that this transition leads to the occurrence of a small percentage of α-helix.

TABLE 3. Estimation of the secondary structural elements of the protein portion of CEA and SP-1

Compound	α-helix	β-structure	β-turn	Disordered form
CEA (pH 7.2, 20°C)	0	69	15	16
CEA (pH 7.2, 80°C)	11	41	17	31
SP-1 (pH 7.2, 20°C)	0	62	33	5
SP-1 (pH 7.2, 70°C)	8	55	29	8

Conformational alterations of CEA (Fig. 2a, b) are reversible with respect to the secondary structure while heating over 55°C gives rise to irreversible conformational alterations in the tertiary structure. In addition, UV spectra of TPDS (thermal perturbation differential spectra) confirmed the evidence of the CD spectra concerning the conformational transition in the CEA molecule (Fig.2c). A calculation showed that three tryptophan residues and 16 tyrosine ones in CEA are accessible to the molecules of solvent under normal conditions (pH 7.2, Δt=5-25°C). Heating the CEA solution to 75°C with subsequent cooling to 25°C resulted in direct and inverse curves (Fig. 2c) which were identical for tyrosine residues only. As for tryptophan, here an inverse situation could be observed; only one residue remains accessible for the molecules of water. This indicates a quite different CEA assembly after thermal denaturation.

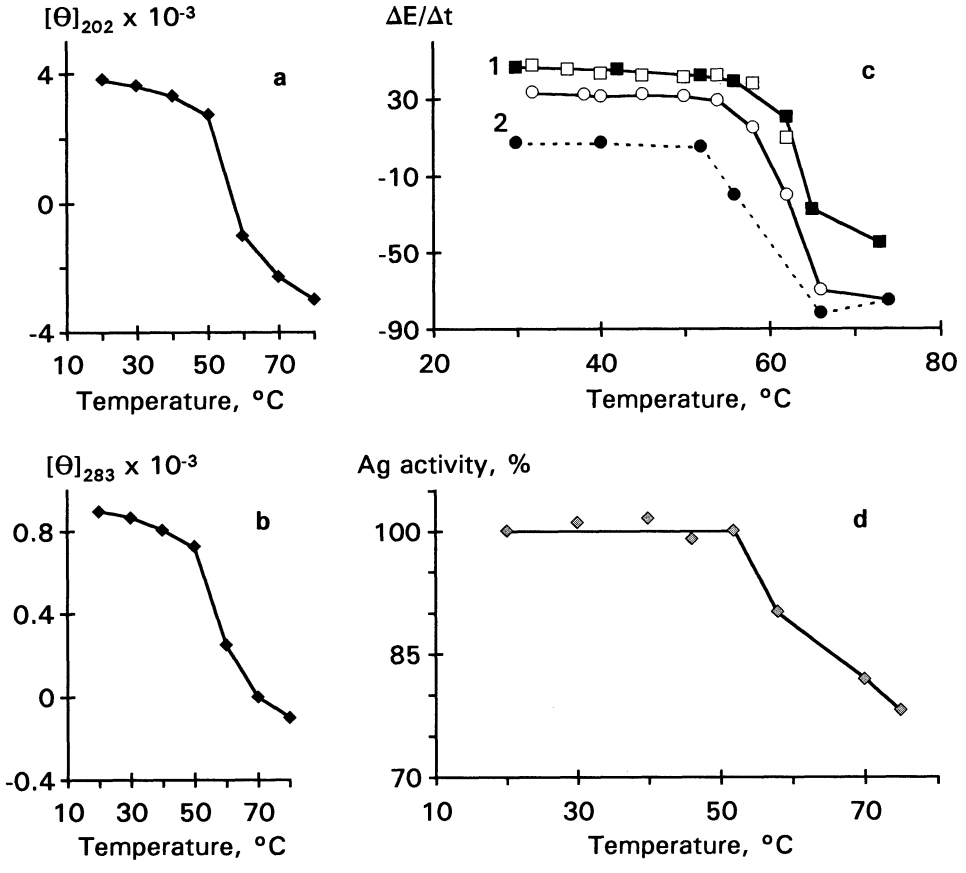

Fig. 2. The influence of temperature on the conformation and antigenic activity of CEA.
a, **b** 202 and 283 nm band ellipticity variations with temperature of solution. **c** Thermal correlation of band amplitudes of tyrosine (1) and tryptophan (2) residues in spectra of thermal perturbation of CEA; bright and dark points correspond to heating and cooling, respectively. **d** Interrelations of the antigen activity and the temperature of prior incubation of the solution.

A comparison of antigenic activity with spectral data (Fig. 2d) indicated that CEA antigenic activity is unchanged up to the conformational transition, while a further rise of temperature (over 55°C) causes a loss of antigenic activity of CEA by 20-25%.

Conformational alterations of SP-1 (Fig. 3a, b) after the thermal denaturation are reversible only in part when the solution is cooled to 20°C. The correlation of antigenic activity data with spectral results (Fig. 3c) indicated that the conformational transition in the SP-1 protein spatial structure causes the antigenic activity to drop as low as 20% relative to the initial level; after 7 days, only 50% of activity is regained.

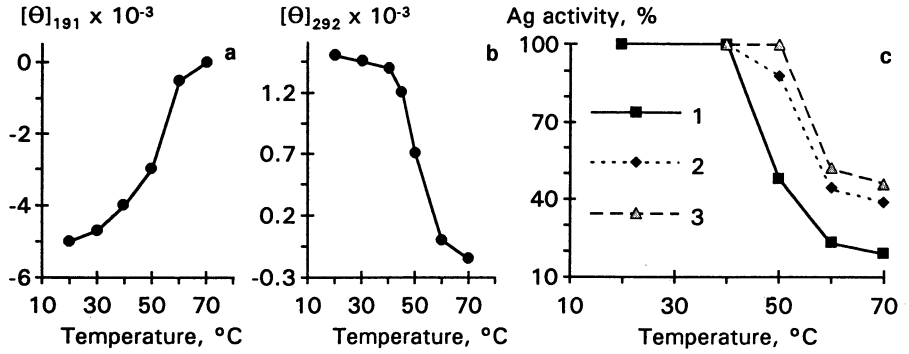

Fig. 3. The influence of temperature on the conformation and antigenic activity of SP-1. **a, b** 191 and 292 nm band ellipticity variations with temperature of solution. **c** Prior incubation temperature effect on SP-1 antigenic activity in solution: 2h at 20°C (1); 2 days at 4°C (2); 7 days at 4°C after incubation (3).

An investigation of the CD spectra of CEA and SP-1 at various pH values showed that a substantial alteration of the CEA conformation occurs at pH values below 3.0 and over 9.5. A complete reduction of the secondary structure takes place under neutralization of acidic (pH < 4) and alkaline (pH > 9) solutes of CEA. Simultaneously, the tertiary structure is reduced to 74% while the CEA antigenic activity decreased to 20% of the initial one. With a pH varied within 4-11, no irreversible changes in the structure of the protein portion of SP-1 or in its antigenic activity have been observed. When neutralizing from pH 2.0, the SP-1 antigenic activity decreases to 30% of its parent, and is seen to recover as the native molecular structure is restored.

The immunodiffusion comparison of heated and pH-denatured CEA and SP-1 specimens has shown their total immunochemical identity with the native antigens, and no molecular degradation has been revealed by SDS electrophoresis. Therefore, the diminution in antigenic activities of SP-1 and CEA is related to an alteration of the spatial structure of their protein portions.

Localization of antigenic determinants

In addition we have carried out a study on the role of the protein portions and the sugar moieties of CEA and SP-1 in the formation of their antigenic determinants. To this end, a series of structurally differing derivatives of CEA and SP-1 have been studied and the chemical modification of tyrosine and tryptophan residues and of the carbohydrate moieties has been performed (Ref. 18, 19).

Deglycosylation of CEA and SP-1 by anhydrous HF results in a substantial alteration of the conformation of the protein portion of both antigens (Ref. 18, 19) accompanied by a complete loss of virtually all the antigenic activity of CEA (Table 4). Such a behaviour of CEA during deglycosylation appears to demonstrate an important role of the sugar moiety in the formation of the spatial structure and of the antigenic determinants of CEA. To the contrary, SP-1 still reacts with its antibodies. The loss of SP-1 antigenic activity seems to be related to an alteration of the spatial structure of the protein portion. A transition of the peptide core structure into a disordered one was observed for CEA treated with 6 M guanidine hydrochloride. A decrease in the ability to bind antibodies demonstrates that some epitopes of CEA are formed as the constituents of its tertiary structural pattern.

An elimination of neuraminic acid residues was shown to affect mainly the tertiary structure of CEA followed by a considerable diminution of the antigenic activity although an insignificant alteration of the conformation occurred in this case (Table 4). This phenomenon appears to be connected to the substantial alterations in the conformation of the carbohydrate chains and their orientation with respect to the protein core. The same treatment of SP-1 results in insignificant changes in protein conformation and does not affect its antigenic activity. Treatment of CEA-ND with β-galactosidase was shown to release about half of the galactose residues in CEA (Ref. 19) and to be accompanied by further rearrangement of the tertiary structure of the antigen. Incidentally, there is virtually no change in the secondary structure of the protein core. A negligible loss of antigenic activity was observed in this case in comparison with the elimination of neuraminic acid residues (Table 4). Thus, the conformation is more important than the primary structure for the manifestation of the antigenic activity especially, at any rate, for the sugar moiety.

TABLE 4. Estimated antigenic activities and structural alterations for derivatives of CEA and SP-1.

Derivative[1]	Antigenic activity, %	Alterations in structure	
		secondary	tertiary
CEA-DG	1	significant	significant
SP-1-DG	11%	significant	significant
CEA-GuHCl	23	significant	significant
CEA-ND	39	insignificant	insignificant
SP-1-ND	102	insignificant	insignificant
CEA-NGD	36	insignificant	insignificant
CEA-POR	3	significant	significant
SP-1-CM	3	significant	significant

[1] Following abbreviations used for antigen derivatives: -DG = antigen deglycosylated with anhydrous HF; -GuHCl = antigen treated with 6M guanidine hydrochloride; -ND = antigen treated with neuraminidase; -NGD = antigen treated with neuraminidase and β-galactosidase; -POR = antigen treated with $NaIO_4$ and reduced with sodium borohydride; -CM = antigen reduced with β-mercaptoethanol and carboxymethylated with monoiodoacetic acid.

Periodate oxidation of CEA followed by a reduction with sodium borohydride was carried out to yield the derivative (CEA-POR) containing around 50% of the sugar residues. CEA-POR was found to possess only 3% of the antigenic activity of the parent CEA (Table 4). It is possible that periodate oxidation of CEA results in a considerable rearrangement of its spatial structure. CD-spectra confirms that the secondary structure of CEA-POR appears to be related to the spatial structure of CEA heated up to 80°C while the antigenic activities of both specimens differ in values (Fig. 2d, Table 4).

The results obtained lend support to the suggestion that antigenic determinants of both antigens possess the main conformational character of the molecules. It seems that the SP-1 antigenic determinants are localized in the protein portion of the molecule while participation of the sugars in the formation and orientation of the carbohydrate chains relative to the protein core of the antigen appears to be important for CEA. To substantiate this in more vigorous terms, we have compared the antigenic activity and spatial organization of both antigens for the modified sugar moiety and some amino acid residues.

For the mild modification of the antigen sugar chains, we have used the ability of sugars to form stable negatively charged complexes with boric acid and its salts. The incubation of SP-1 in a borate buffer led to a 10% increase in antigenic activity as compared to the antigen, incubated in a phosphate buffer. The immunoenzyme assay calibration curves for SP-1 in the borate and phosphate buffers run parallel to each other, and CD spectra of the two solutions were identical. This provides evidence that borate complexes, formed in the sugar chains, do not affect the SP-1 protein portion spatial structure, and the observed slight increase in antigenic activity can be associated with conformational changes in the sugar chains. Presumably, the conformational rearrangement of the SP-1 sugar chains renders certain portions of the SP-1 molecular polypeptide framework more accessible to the antibodies. A treatment of CEA with borate buffer led to a decrease to 5% of the initial antigenic activity. Removal of borate resulted in a complete recovery of the antigenic activity. CD spectra failed to reveal alterations of the secondary and tertiary structures of the protein portion of CEA in borate buffer. Therefore, sugar residues appear to be constituents of the topographic antigenic determinants of CEA.

Modification of tryptophan residues showed that the antigenic determinants of CEA fail to contain tryptophan residues on their surface (Ref. 21). The internal tryptophan residues appear to play an important role in the formation of the native spatial structure of the protein portion. A direct correlation of the antigenic activity with alterations of the spatial structure of the protein portion was observed. As shown by CD spectra (Ref. 22), the modification of only one surface tryptophan residue produces no change in the SP-1 native structure; still, the SP-1 antigenic activity is seen to drop by 26%. The modification of another and subsequent tryptophan residue causes a distinct degradation of the SP-1 protein portion native structure and significantly reduces its antigenic activity. These results indicate that at least one surface tryptophan residue is involved in the SP-1 antigenic determinants. The changes in the SP-1 protein portion spatial structure, produced by further modification of the tryptophan residues, precludes an unambiguous elucidation of the extent of involvement of these moieties in the formation of the SP-1 antigenic determinants, but provides supplementary support to the suggestion that the determinants are mainly topographic in character.

In a similar manner, the modification of three tyrosine residues leads to changes of the protein portion spatial structure and reduces the antigenic activity of the modified specimen by 30%.

Still, these data fail to permit us to conclude with certainty that the tyrosine residues are constitutionally included in the SP-1 antigenic determinants, since the observed changes in antigenic activity may be due to rearrangements in the spatial structure of the SP-1 molecule protein portion.

Half of the antigenic activity of CEA was observed in the case of the modification of all twelve histidine residues or of half of them. Although modification of histidine residues causes slight alterations of the spatial structure of the protein portion, a loss of 50% of the antigenic activity fails to be correlated with either the alterations of the CEA conformation or an occurrence of histidine residues as epitope constituents. It is more probable that this modification results in alterations of orientation of carbohydrate chains in relation to the protein core. This suggestion is in good agreement with the data obtained by desialylation of CEA.

Biological role

Although CEA and SP-1 have been discovered more than 20 years ago, the biological functions of these antigens are still unknown. Some progress was made during these last years with respect to CEA after finding the striking sequence homology between CEA and the proteins of the immunoglobulin family. These proteins include immunoglobulins, growth factor receptors, T-cell antigens, intercellular adhesion molecules and the class I and II of major hystocompatibility complex (MHC) glycoproteins. It is supposed that CEA may play an important role in cell-cell interactions (Ref. 23). CEA is a membrane anchored glycoprotein possessing an extremely high content of carbohydrates and consequently may participate in cell recognition phenomena. It was shown that the CEA family proteins may function as cell adhesion molecules *in vivo* (Ref. 7).

Quite in contrast to this progress little is known about SP-1. This antigen is produced in the trophoblast and secreted in large amounts into circulation during pregnancy. More recently we have found[1] that SP-1 exhibits phosphatase activity on both low- and high-molecular weight DNA substrates. It was shown that SP-1 is capable to split the phosphoester bonds in γ-[^{32}P]-ATP, p-nitrophenylphosphate and 4-nitrophenyl-5'-TMP. SP-1 was also found to effectively hydrolyse [^{3}H]-poly(U) and [^{3}H]-DNA of embryos of the marine urchin *S.intermedius*. The nuclease activity of SP-1 was also tested with DNA plasmid pBR322. Using the EcoRI/EcoRV fragment of DNA plasmid pBR322 as a substrate we have demonstrated that enzymic hydrolysis proceeds from the 3' end of the DNA chain by exonuclease's type. Our findings as well as the significant increase of SP-1 serum concentration during pregnancy and trophoblastic deseases allow to propose that SP-1 may function as a regulatory factor which promotes the activation and proliferation of certain cells. This proposition is supported by the fact that SP-1 was found in secondary placental cells and human fibroblasts after many passages (Ref. 24). It may indicate its association with growth and maintenance of non transformed cells *in vitro*. It should be emphasized that CEA was not shown to possess SP-1 enzymic properties.

AN INVESTIGATION OF ONCOPRECIPITINS

Nowadays, the causes of neoplasm origin in the vertebrates are more investigated than those in the invertebrates in which the recorded neoplastic protection mechanisms are either lacking or markedly underdeveloped. In the same time, the invertebrates appear to possess a simple and rather effective defense against somatic mutations, judging by the extremely low incidence of malignant tumors in invertebrates. It may be supposed that special substances blocking the activity of oncogens occur in them.

Recently, a new type of natural substances termed oncoprecipitins has been discovered by us in some marine invertebrates (Ref. 8, 9). Oncoprecipitins were shown to represent substances which interact specifically with oncofetal antigens in antigen-antibody manner. The following oncoprecipitins: crustacin from the hermit crab *Pagurus prideauxii* and cyprein from the cowrie *Cyprea caputserpentis*, reacting with CEA and oncoprecipitin A from ascidian *Didemnum ternatum* interacting with SP-1 have been isolated. In this part of our paper the results of the immunochemical and physico-chemical studies of these oncoprecipitins and also examples of their application are presented below.

Immunochemical studies of oncoprecipitins

On immunochemical comparison (Fig. 4a, b), crustacin reveals a complete and cyprein a partial identity with the antibodies against CEA. Therefore, CEA possesses similar antigenic

[1] Moroz, S.V., Gafurov, Yu.M., submitted for publication

determinants for binding of crustacin and antibodies against CEA. The full immunochemical identity was revealed by the reaction between oncoprecipitin A, SP-1 and its antibodies (Fig. 4c).

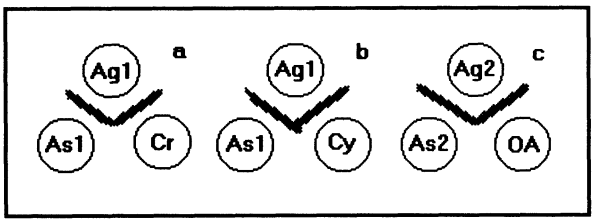

Fig. 4. Immunochemical comparison of oncoprecipitins with the antigen test systems. **a, b** Comparison of crustacin and cyprein with the CEA test system: Ag1 = CEA; As1 = antiserum against CEA; Cr = crustacin; Cy = cyprein; **c** Comparison of oncoprecipitin A with SP-1 test system: Ag2 = SP-1; As2 = antiserum against SP-1; OA = oncoprecipitin A.

Numerous lectins, known as being reactive towards CEA, exhibit total immunochemical identity with the antibodies against this antigen (Ref. 25, 26). In addition, the lectins are also capable of reaction with other glycoproteins containing carbohydrate chains structurally related to those of CEA. Oncoprecipitins, as distinct from lectins, were shown to be reluctant toward interaction with the glycoproteins from normal human organs and tissues, as well as with a number of glycoproteins containing carbohydrate chains structurally similar to those of CEA and SP-1 (Fig. 5, Ref. 9, 27), for example, NCA-1, which is a member of CEA subgroup (Ref. 3). Crustacin and cyprein were found to give precipitation bands with a desialylated CEA, and with the latter after removal of 2/3 of the galactose residues and some mannose residues. However, it should be noted that cyprein exhibited a partial identity with α_1-acid glycoprotein (AGP) and full identity with NCA-1 (fig. 5 b). In addition, oncoprecipitin A showed a diffuse precipitation band with an unidentified constituent of blood sera of humans, rabbits and mice (Fig. 5c). Still, admitting the validity of the recently enunciated hypothesis on a relationship between CEA and AGP (Ref. 28), it may be presumed that cyprein interacts with antigenic determinants common to these two antigens.

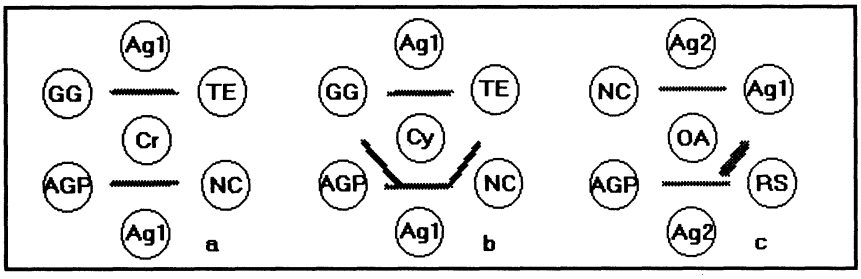

Fig. 5. Immunochemical comparison in test system antigen-oncoprecipitin for various bioglycans. **a** Test system CEA-crustacin: Ag1 = CEA; Cr = crustacin; AGP = α_1-acid glycoprotein; GG = galactan from ginseng; NC = NCA-1; TE = combined extracts of human normal organs and tissues; **b** Ag1, AGP, NC, TE, GG = same as in **a**; Cy = cyprein; **c** Ag1, AGP, NC = same as in **a**; Ag2 = SP-1; OA = oncoprecipitin A; SR = rabbit serum.

Interaction between SP-1 and oncoprecipitin A fails to be inhibited with the sugars involved in the carbohydrate chains of SP-1. In contrast to SP-1, an interaction of oncoprecipitin A with a normal blood serum component was found to be inhibited with N-acetyl-D-glucosamine.

We have performed sugar inhibition of the CEA-oncoprecipitin binding reaction to demonstrate that the interaction of crustacin and cyprein with CEA is as specific as the homologous antibodies with CEA. It was estimated by competitive immuno enzyme assay that CEA-crustacin binding is inhibited by numerous monosaccharides (Ref. 29), including those not occurring in CEA. As a common feature, it may be noted that the inhibition of CEA-oncoprecipitin interaction by sugars was distinctly less specific as compared to that involving lectins. It should be emphasized that the 50% inhibition of CEA binding with oncoprecipitins was affected at relatively high concentrations of sugar, while 100% inhibition could not be accomplished even at sugar concentrations as high as 0.5 mol/l. Certain glycoproteins possessing carbohydrate chains structurally related to those of CEA were not inhibitory toward the interaction of CEA with the oncoprecipitins studied.

A high affinity of crustacin and cyprein toward CEA has further been evidenced by experiments of 50% inhibition with CEA of the binding of the antigen with crustacin and cyprein (Fig.

6). Such an inhibition was accomplished at CEA concentrations of 1.7×10^{-10} and 1.1×10^{-11} *M*, respectively. These values are close to those common for the antibodies against CEA. An additional piece of evidence is also provided by the association constants for antigen-oncoprecipitin complexes; the respective values of 10^{-8} and 10^{-9} were also close to those characteristic of high-affinity antibodies.

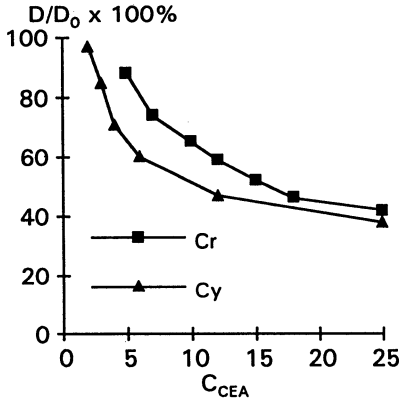

Fig. 6. CEA concentration effect on the binding reaction in the CEA-oncoprecipitin systems; Cr = CEA- crustacin system; Cy = CEA-cyprein system; C_{CEA} = concentration of added CEA $x10^{-9}$ *M* for CEA-crustacin system and $x10^{-10}$ *M* for CEA-cyprein system.

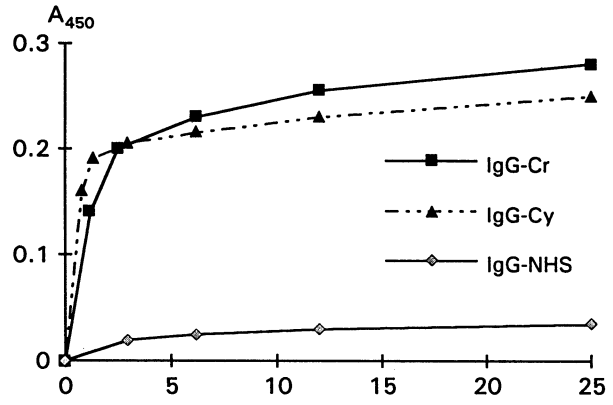

Fig. 7. Determination of immunochemical activity of CEA using immunoglobulins against crustacin (IgG-Cr), cyprein (IgG-Cy) and normal human serum (IgG- NHS).

The interacting surfaces of antigen and antibody are well known to correspond to each other as "key-to-lock". Ierne et al. (Ref. 30) showed that knowing the configuration of the present antibody as "a lock", a new antibody molecule may be obtained from other antibody-like substances. This new molecule was proved to possess the sites with a configuration of the parent antigen as "a key". This is a general principle for obtaining anti-idiotypic antibodies. We have prepared immunoglobulins by immunization of rabbits with crustacin and cyprein and demonstrated that the immunoglobulins obtained were related to the parent anti-CEA antibodies and contained the same antigenic determinants as CEA. In order to confirm this statement, the immunoglobulins obtained were used instead of CEA in an immunoenzymic assay using rabbit IgG against CEA (Fig. 7). We have found that a specific interaction was observed with both immunoglobulins prepared. The interaction failed to be observed in the control tests using normal human, rabbit and ass sera. Thus, a higher complementarity of crustacin and cyprein to the specific sites of CEA was confirmed by data indicating a higher specificity of the oncoprecipitins to CEA. It should be emphasized that the analogous experiments to obtain the sites of immunoglobulins which were complementary to antigenic determinants of the antigen used for immunization have been realized earlier on idiotypic antibodies only. Our experiments were the first ones to demonstrate the possibility of obtaining on immunoglobulins the sites which are complementary to the specific ones of the antigen by using substances of non-immunoglobulin behaviour.

Physico-chemical properties

All oncoprecipitins studied appeared to be glycoproteins with relatively small molecular masses and with a high protein content. As evidenced by SDS electrophoresis (Table 5) and by the determination of N-terminal amino acid sequence, crustacin consists of two similar subunits, bound by disulphide linkages while oncoprecipitin A consists of four similar subunits. Crustacin and cyprein were distinguished from oncoprecipitin A by an enhanced lability and could be easily converted into immunochemically inactive forms. In addition, the CEA specific precipitation with crustacin and cyprein was shown to increase in the presence of calcium and magnesium ions.

Using CD and fluorescence spectroscopy we have studied the effect of temperature, ion strength, pH and calcium ion concentration on crustacin spatial structure. The calculation of crustacin secondary structural elements showed the presence of 3% α-helix, 84% β-structure, 14% β-turn and the complete absence of the disordered form. At 45-50°C an irreversible conformational transition of the crustacin protein portion accompanied by the loss of the CEA-binding activity has been found. Crustacin

TABLE 5. Physico-chemical properties of oncoprecipitins

Property	Crustacin	Cyprein	Oncoprecipitin A
Molecular mass, as determined by SDS-electrophoresis, for			
Intact protein (kDa)	36 ± 1	44 ± 2	36 ± 2
β-Mercaptoethanol-reduced protein (kDa)	17 ± 1	46 ± 2	9 ± 1
Electrophoretic mobility in agar (pH 8.6)	α-globulins	β,γ-globulins	α-globulin
Molar extinction (M^{-1} cm^{-1})	70,000	48,400	62,800
N-terminal amino acid	Phe	Ser	Ala
Protein (%)	87	82	97
Neutral monosaccharides (%)	3.0	14.0	2.1
Hexosamines (%)	0.0	3.6	0.0

was shown to be a calcium-binding protein and the stability of its native conformation is being markedly enhanced by calcium ions (1 mM Ca^{2+} shifted up the transition temperature by 10°C). Calcium binding and ionic strength increase lead to alteration of both secondary and tertiary structures of crustacin. The elimination of calcium ions caused the drop of crustacin CEA binding activity to 60% while heating the solution up to 50°C resulted in its complete loss.

Titration of crustacin with sugars within concentrations of 1/10 to 1/1,000 failed to give alterations in UV, CD and fluorescence spectra (Ref. 31). This phenomenon indicates an absence of specific interactions of sugars with crustacin since an interaction of lectins with specific sugars is accompanied by a peculiar alteration of UV, CD and fluorescence spectra.

Therefore, the evidence obtained enable us to conclude that the oncoprecipitins studied exhibit a high specificity toward CEA and SP-1. Viewed in terms of specificity, the interaction between oncoprecipitins and their antigens is distinct from the reaction of lectins with glycoproteins as well as with CEA and SP-1, but bears a resemblance to antibody-antigen interaction.

Examples of applications on oncoprecipitins

We have applied specific test system CEA-crustacin for the quantitative determination of CEA in various extracts of tumors and normal human tissues. The results obtained were compared with those of the CEA - anti-CEA test system (Table 6, Ref. 32). Both test systems were found to react very

TABLE 6. Estimation of CEA in extracts from tumors and normal human tissues.

Tumor-affected organs	CEA - anti-CEA			CEA-crustacin		
	total number of tests	positive tests	% positive	total number of tests	positive tests	% positive
Intestine	32	22	68.3	32	18	56.3
Stomach	27	12	44.4	27	22	81.5
Lung	24	3	12.5	24	4	16.7
Urogenital system	13	0	0	13	0	0
Mammary gland	20	1	5	20	0	0
Kidney	23	0	0	23	0	0
Ovary	21	2	9.5	21	6	28.6
Uterus	2	0	0	2	0	0
Bones	20	0	0	20	0	0
Soft tissues	20	1	5	20	0	0
Brain	20	0	0	20	0	0
Organs in adult man	28	2[1]	7.1	28	2[2]	7.1
Organs in human fetus (6-12 weeks)	14	0	0	14	0	0
Organs in human fetus (24 weeks)	29	4	13.8	29	4	13.8

[1] Concentration was 40 mg/ml (relative to protein). [2] The same individuals.

closely except for cases of stomach and ovarian tumors, in which the CEA-crustacin test system appeared to exhibit a higher sensitivity toward CEA.

An application of crustacin for immunohistological study of various human tumors and normal and embryonal tissues (Ref. 33, 34) confirmed the results of the immunodiffusion tests. The reaction with crustacin has a more specific character than the one with anti-CEA antibodies, and is more expressed in ovarian tumors.

We have studied an action of oncoprecipitin A and SP-1 on the transformed cell lines A-431 and HeLa-M. As was mentioned above, SP-1 is synthesized by placental syncytiotrophoblasts and secreted in large amounts into maternal circulation. Its concentration in serum increases gradually during pregnancy and reaches a very high level at term, indicating its important role for pregnancy maintenance. SP-1 quantitative determination during pregnancy may be used for surveillance of pregnancy development. Also, SP-1 is found in the sera of patients with hydatidiform mole, invasive mole and choriocarcinoma. *In vitro* it is produced by placental cells, non-trophoblastic tumor cells, human fibroblasts and by epidermoid cervical cancer cells (Ref. 24, 35, 36). Recently, we have found (Ref. 37) that the peripheral blood lymphocytes of donors and of patients with various hemoblastosis were capable to bind anti-CEA and anti-SP-1 antibodies. This phenomenon indicates that the peripheral blood lymphocytes possess binding sites common with those of CEA and SP-1. The HeLa is known to be a cell line derived from the human uterine cervical cancer, and the A-431 culture is a human epidermoid carcinoma cell line. Therefore these cellular cultures appeared to be suitable tumor targets for oncoprecipitin A. An action of SP-1 on tumor cells caused an alteration of their actin cytoskeleton (Ref. 38). Incubation of tumor cells with oncoprecipitin A was accompanied by increased cell size and morphology. Intensively coloured clusters of actin microfilaments were observed in the perinuclear zone of the treated cells but not in intact cells. The elimination of oncoprecipitin A from the culture medium following incubation with SP-1 resulted in recurrent cytoskeleton changes in HeLa cells: the final cells resembled the intact cells in morphology and in distribution of actin microfilaments. The same treatment of A-431 cells was shown to cause a concentration of actin bundles around the cells.

Although, the biological functions of oncoprecipitins are unknown up to now, their high specificity to oncofetal antigens allows us to suggest that they appear to play an important role in surveillance of neoplastic growth and to be an unusual defence of invertebrates against cancer. This suggestion is confirmed by a low frequency of malignant tumors in invertebrates and by a phenomenon that CEA represents the most common marker of neoplastic growth. Indirect confirmation is connected with the appearance of receptor specific molecules at the earliest evolution steps as constituents playing an important role in the processes of cell contacts and differentiation which, as known, are interconnected with the process of neoplastic transformation (Ref. 39).

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