Formulations Based on Carboxymethyl-starch:Iodine Complexes

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Abstract: There is a major need for novel approaches to limit contamination with respiratory viruses such as SARS-CoV-2 (Covid-19) and influenza. Since viral transmission is by aerosols, the oral and nasopharyngeal decontamination seems of relevance to prevent virus spreading. Carboxymethylstarch (CMS), microspheres of CMS cross-linked with Sodium TriMetaPhosphate (STMP), and high amylose starch cross-linked (CL) with epichlorohydrin HAS-CL were proposed as matrices for iodine inclusion complexes as anti-infective agents. By hydrolysis of CMS matrix with salivary alpha-amylase, the released iodine would act against infective agents. The iodine complexation induced some structural modifications on the CMS network. CMS:Iodine presented enhanced mucoadhesion, a markedly increased sprayability, and a higher susceptibility at amylolysis with alpha-amylase, when compared to unloaded CMS.

Keywords: carboxymetylstarch iodine complexes, mucoadhesion, spray, antiviral agents

(Title and abstract in Romanian)

Formulari pe baza de complecși de Carboximetil amidon

Rezumat: Există o nevoie majoră de noi abordări pentru a limita contaminarea cu virusuri respiratorii precum SARS-CoV-2 (Covid-19) și gripa. Deoarece transmiterea virală

se face prin aerosoli, decontaminarea orală și nazofaringiană pare a fi relevantă pentru a preveni răspândirea virusului. Carboximetilamidonul (CMS), microsfere de CMS reticulate cu Sodiutrimetafosfat (STMP) și amidon cu conținut ridicat de amiloză reticulată (CL) cu epiclorhidrină HAS-CL au fost propuse ca matrici pentru complec*și* de incluziune a iodului ca agenți antiinfecțioși. Prin hidroliza matricei CMS cu alfa-amilază salivară, iodul eliberat poate acționa împotriva agenților infecțioși. Complexarea iodului induce unele modificări structurale asupra rețelei polimerice a CMS. CMS:Iod prezinta a mucoaderență îmbunătățită, o pulverizare semnificativ crescută și o susceptibilitate mai mare la amiloliză cu alfa-amilază, în comparație cu CMS necomplexat cu iod.

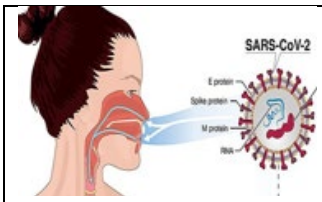
Cuvinte cheie: complecși amidon:iod, mucoadeziune, spray, actiune antivirala.

Introduction

Coronavirus disease 19 (COVID-19), caused by the highly transmissible and pathogenic SARS-CoV-2 virus, has led to the global pandemic. While various vaccines have been developed, drugs against SARS-CoV-2 have been primarily focused on antivirals/antimicrobials administrated by injectable forms. Despite the peaks of pandemia have passed, there are still concerns about a new wave or long COVID-19 affecting many people that can have a wide variety of symptoms that can range from mild to severe and may be like symptoms from other illnesses. These symptoms can persist weeks, months, or years after COVID-19 illness and can emerge, resolve, and reemerge over different lengths of time. Prevention from the very first symptoms may be a very simple and efficient solution. Using a spray with virucidal activity applied directly on the area of mouth and throat can inactivate the virus and eliminate the consequences of infection (Fig. 1).

In this study, we are proposing new formulations with virucidal activity that are based on carboxymethylstarch:iodine complexes (CMS:I_2). By hydrolysis with salivary amylase, CMS:I_2 liberates iodine exerting a strong virudical activity against human coronavirus hCoV-OC43 - an accepted model mimicking the SARS-CoV-2 virus.

Starch and its derivatives have been widely used in the formulation of a variety of drugs to modulate their release patterns [1]. In this study, carboxymethyl starch iodine inclusion complexes were prepared by aqueous procedures and proposed as new spray formulations to be applied as anti-infective treatment. The hypothesis was that CMS, grafted with polar carboxylic groups, may present expanded V-helices, helping to load higher amounts of iodine.



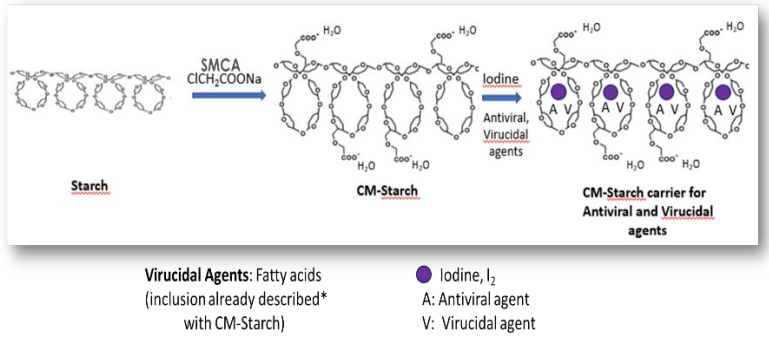


Figure 1. CM-Starch expanded V-helix and inclusion of Iodine (I_2): antiviral and virucidal agents

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Methodology

1. Reagents

Hylon® VII, a high amylose corn starch (HAS, 70 % of amylose), was from Ingredion (Westchester, IL, USA). Sodium monochloroacetate (SMCA), sodium trimetaphosphate (STMP), epichlorohydrin, mucin, gelatin, bacterial α-amylase and human salivary α-amylase were from MilliporeSigma (Darmstadt, Germany). Iodine, KI, NaOH, and HCl were from Fisher (Hampton, New Hampshire, USA).

2. Starch derivatization

2.1. *Preparation of carboxymethyl starch (CMS) powder*: an amount of 140 g of Hylon VII (corn starch with a 70 % amylose content), was first suspended in approximatively 340 mL of water at 55 ◦ C for 20 min under stirring and then an equal volume of 360 mL of 1.5 M NaOH was added with continued stirring for 1 h. Carboxymethylation was carried out by adding 50 g sodium monochloroacetate (SMCA) continuing the reaction for 2 h in the same conditions of stirring and 55 ◦C [1].The obtained CMS derivative presented a degree

of substitution (DS = 0.15 ± 0.030) and was hereto called CMS(DS 0.15). Another CMS derivative was obtained exactly in the same conditions but doubling the amount of SMCA from 50 g to 100 g and obtaining a CMS product with DS = 0.30 ± 0.080, hereto called CMS(DS 0.30).

2.2. *Preparation of microspheres CMS-MS* was done by a water-in-water (W/W) emulsion method in three steps [2]: a) preparation of the continuous phase; b) addition of the dispersed phase: the emulsion; c) cross-linking of the beads: for CMS (DS 0.15) crosslinking, 1.0 g, 1.5 g, or 2.0 g STMP were added to the emulsion under continued stirring for 30 min, to obtain respectively different cross-linking degrees conventionally expressed as percentages of crosslinker (20 %, 30 % and 40 %) used to cross-link the CMS (DS 015) materials. The cross-linked CMS derivatives are hereto called CMS-CL20, CMS-CL30 and CMS-CL40, where the numbers represent the percentage of STMP cross-linker reported to the initial amount of CMS to cross-link. The detailed method of MS drying was described previously [2]. The obtained dry CMS-CL20(MS), CMS-CL30(MS) and CMS-CL40 (MS).

2.3. *Iodine inclusion:* Two methods were tested, for the inclusion of iodine in starch: i) with a Lugol's reagent (solution) and ii) by iodine vapor. i) Inclusion of iodine using Lugol's reagent: iodo-iodide solution (I2 + KI) was prepared in the laboratory (10 g KI and 5 g I_2 in 100 mL water), allowing the inclusion of iodine molecules in the helix structures formed by the starch (iodine retained by Van der Waals bonds). ii) Iodine vapor: 1 g powders of starch derivatives were placed in a beaker containing 2 g of solid molecular iodine I_2 and the system was maintained in a desiccator for one week, checking the mass of each sample at the end.

2.4. *Mucoadhesion and sprayability assays*: A mucin-gelatin adhesive supports were prepared by casting from a warm filmogenic solution containing 10 % gelatin and 5 % mucin. The adhesion of suspended particles was tested by pipetting 100 µL of the suspensions of samples onto the mucin or mucin-gelatin plate on the hexagonal weighing dishes containing mucin-gelatin support. For each material, three samples were applied for different times of contact (t =0 s; 120 s and 300 s) to the same support at distance of 10 mm each other. At the end of the interval, the adhesive backing was immediately placed at 45◦ inclination (time zero) and the time taken for droplets to flow 20 mm down onto the mucin-gelatin surface was recorded and considered to evaluate the relative mucoadhesion of various formulations (Fig. 2) [3]. To test the sprayability of the liquid formulation CMS (DS 0.15)):I_2, the suspension was first homogenized and loaded into a manual spray bottle. The test was performed on a white paper placed to assess the spray distribution. The spray bottle was held approximately 15 to 30 cm from the surface, and an intermittent spray was applied. For sprayability, CMS(DS 0.15):I_2 suspensions 1 % and 5 % were used to estimate the drop

profile splashed on a white paper screen. The sprayed drop pattern was compared to the commercial PVP-I (Betadine) at same concentrations.

2.5. *Hydrolysis of iodine complexes by different α-amylase preparations.* Commercial human salivary α-amylase, bacterial α-amylase, or crude saliva were assayed by adding 1 mL of each enzyme stock solution (the enzyme concentration were chosen such as to obtain 0, 20, 40 and 60 enzyme units) to 1 mL of 1 % (w/v) Hylon VII, CMS, or CMS:I_2 suspension. The amylolysis with liberation of reducing sugars was carried out by incubating of the mixture at 37 °C for 3 min. The reaction was stopped by adding 1 mL of 3, 5-dinitrosalicylic acid and heating it at 100 °C for 5 min for determination of reducing sugars [4]. One enzyme unit (EU) of enzyme) is defined as the amount of enzyme that releases 1 μ mol of reducing sugar (i.e. maltose equivalent) per minute under the standard assay conditions.

Results

Iodine loading of CM-Starch derivatives

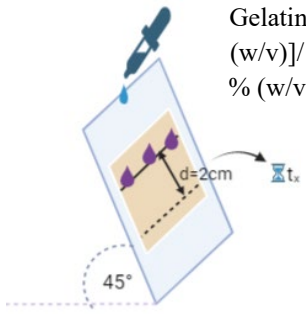
The highest iodine contents (mg/g of starch derivative powder) were for cross-linked High Amylose Starch (HAS-CL10): 95.71 ± 5.1 mg/g, for CMS (DS 0.15): 90.45 ± 2.4 mg/g and for CMS-CL40(MS): 108.27 ± 1.1 mg/g. A higher iodine loading of 95.71 mg/g for HAS-CL10 (the higher degree of cross-linking with epichlorohydrin) than that of 64 mg/g for HAS-CL5 (the lower degree of cross-linking, with only 5 % epichlorohydrin) was an unexpected behavior. Normally, a higher crosslinking could prevent the access iodine to the V-helix of the starch matrix. In our case, the explanation resides on previous observations [5,6] showing that denser cross-linking bridges with epichlorohydrin (8.4 Å) would hinder the formation of hydrogen bonds (4.1 Å) and so will prevent a tight interchain association of cross-linked (HAS-CL), acting as a spacer and affording better access of iodine to its V-helices. The iodine loading (90.45 mgI_2/g) of carboxymethylated material CMS (DS 0.15) with a low degree of substitution (DS 0.15) was higher than that of 18 mg/g of CMS (DS 0.30) with a higher DS. A better fitting of hydrophobic iodine size with the size of the hydrophobic channel of V-type helix cannot be excluded. In the CMS microspheres (MS) cross-linked (CL) with STMP, that the iodine loading increased linearly with the cross-linking degree (cld). The highest loading (108.27 mgI_2 /g) was found at the greatest cld: CMS-CL40 (MS). This can be explained by the previous observation that carboxymethylation obtained by grafting of polar groups would enhance the diameter of the V-helix [6].

The CMS (DS 0.15) exhibited the best iodine retention. This spontaneous reaction is a particularly advantageous loading procedure, taking advantage of i) the property of I_2 to

sublimate and ii) the expanded V- helix form of CMS (DS 0.15) starch. Best iodine inclusion was found only with modified starches presenting expanded V-type helices.

Unexpectedly, CMS:I_2 presents a higher susceptibility at amylolysis and enhanced mucoadhesion and sprayability (Table I).

Figure 2: Mucoadhesion test



Gelatin solution [20% (w/v)]/ and mucin [10 % (w/v)] in a ratio 1/1

Table I: The effect of iodine loading on mucoadhesion, viscosity and sprayability for CMS

Samples	Viscosity	Sprayability
CMS 1%	10.7	N.D
CMS:I_2 1%	1.15	++++
CMS 5%	27.7	N.D
CMS:I_2 5%	3.9	+++
CMS-MS(CL 40%) 1%	0.52	N.D
CMS-MS(CL 40%):I_2 1%	0.66	++
BETADINE® 1%	0.93	+++
BETADINE® 5%	1.43	+++

It was of interest to evaluate the mucoadhesive characteristics of CMS:I complexes in view of further utilization for buccal and oropharyngeal. A much stronger mucoadhesion measured as a longest runtime (480s) was found for CMS (DS 0.15):I_2 formulations when compared with the commercial product Betadine. A possible explanation of higher mucoadhesion for CMS(DS 0.15):I_2 can be related to the nonpolar iodine hosted by the V-helix, that would trigger a repulsion of outer carboxylic groups, enhancing thus the adhesion. An increase in mucoadhesion was found with all starch materials when loaded with iodine (Table 1), important feature for further applications of CMS(DS 0.15):I_2 complexes as microbicidal agents. The unexpected decrease of viscosity for loaded CMS(DS 0.15)I_2, can be explained by the non-polar iodine inside the V-helix, generating repulsive interactions with outer polar carboxylic groups, enhancing the overall hydrophilicity and decreasing the viscosity.

The amylolysis rates with various alpha-amylases were determined against 1 % Hylon VII, or CMS(DS 0.15), or CMS(DS 0.15):I at various enzyme concentrations: 20–60 U/mL for both commercial salivary and bacterial α 2 -amylases) or with 0.2–1.0 mL for crude saliva. The amylolysis rates were quantified by production of reducing sugars (mainly maltose) at 37 ˚C. The highest amount of reducing maltose equivalent sugars was liberated from CMS (DS 0.15)I_2, followed by CMS (DS 0.15) and Hylon VII, respectively. A similar behavior was observed with these starches treated with crude saliva (data not shown).

Conclusion

Obtained starch derivatives CMS with a degree of substitution (DS) between 0.15 and 0.30, microspheres cross-linked with STMP CMS-CL(20,30.40%)(MS), and starch cross-linked with epichlorohydrin HAS-CL(5,10) were used as polymeric carriers of iodine. This spontaneous inclusion of iodine was found for CM-starch only and has not occurred with non-derivatized starch. It appeared that iodine complexation induced some structural modifications on the starch network. The CMS:I_2 showed high mucoadhesion and a remarkable sprayability, favoring the oropharyngeally administration.

It is suggested that CMS:I_2 formulations could prevent the initial phases of SARS-CoV-2 infection possibly by interfering with viral attachment and entry into target cells.

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