

CHARACTERIZATION OF THE ADSORPTION PROCESS ANALOGOUS PEPTIDES ON ALUMINA GEL

CARACTERIZACIÓN DEL PROCESO DE ADSORCIÓN DE PÉPTIDOS ANÁLOGOS SOBRE GEL DE ALÚMINA

CARACTERIZAÇÃO DO PROCESSO DE ADSORÇÃO PEPTÍDEOS ANÁLOGOS EM GEL ALUMINA

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ABSTRACT

Peptide antigen adsorption on aluminum hydroxide gel must be characterized when formulating vaccines. In this work a peptide belonging to the amino-terminal region of *Plasmodium falciparum* Merozoite Surface Protein and its analogues have been characterized. The adsorption of 17 analogues on aluminum hydroxide which had greater than 10 mmol/L solubility was quantified at 298 K. Adsorption capacity and affinity constant parameters were calculated by applying the Langmuir's adsorption model.

The results have been presented in three groups according to adsorption isotherm trajectory. The first group consists of analogues where the first organization of peptide molecules was presented at low concentrations, followed by a rapid increase in adsorption to high concen-

trations. The second group consists of analogues having an adsorption pattern showing the formation of a first layer at low peptide concentrations and a second layer at greater concentrations. The third group contains analogues whose adsorption involved the formation of two simple layers, this being differentiated from the second group in that after the second layer had been completed, the amount adsorbed grew notably with increased concentration.

The results revealed a more complex pattern that monolayer or bilayer formation. This work constitutes the first approach towards establishing an adsorbed layer structure model using a peptide system.

Key words: Adsorption, aluminum hydroxide, peptide, vaccine, *Plasmodium falciparum*.

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RESUMEN

La adsorción de un antígeno peptídico sobre gel de hidróxido de aluminio debe ser caracterizada para la formulación de vacunas. En este trabajo se caracterizó la adsorción de un péptido que pertenece a la región amino-terminal de la proteína de superficie del merozoite de *Plasmodium falciparum* y sus análogos. Se cuantificó la adsorción a 298 K sobre hidróxido de aluminio de 17 análogos con una solubilidad mayor de 10 mmol/L. Los parámetros de capacidad de adsorción y constante de afinidad se calcularon aplicando el modelo de adsorción de Langmuir.

Los resultados se presentan en tres grupos, de acuerdo con la trayectoria de la isoterma de adsorción. El primer grupo consta de los análogos que presentaron la primera organización de las moléculas de péptido en concentraciones bajas, seguida de un rápido incremento de la adsorción a altas concentraciones. El segundo grupo de análogos tiene un patrón de adsorción que muestra la formación de una primera capa a concentraciones bajas de péptido y una segunda capa a concentraciones mayores. El tercer grupo contiene los análogos cuya adsorción muestra la formación de dos capas simples y se diferencia del segundo grupo en que después de la segunda capa, la cantidad adsorbida crece notablemente con el aumento de la concentración.

Los resultados revelaron un patrón de adsorción más complejo que la formación de monocapa o bicapa. Este trabajo constituye la primera aproximación hacia el establecimiento de un modelo de estructura de la capa adsorbida en un sistema peptídico.

Palabras clave: adsorción, hidróxido de aluminio, péptidos, vacunas, *Plasmodium falciparum*.

RESUMO

A adsorção de um antígeno peptídico sobre um gel de hidróxido de alumínio deve de ser caracterizado para a formulação de vacinas. Neste estudo foi caracterizada a adsorção de um peptídeo que pertence à região amino-terminal da proteína de superfície do merozoite de *Plasmodium falciparum* e seus análogos. Foi quantificada a adsorção a 298 K sobre hidróxido de alumínio de 17 análogos com uma solubilidade maior de 10 mmol/L. Os parâmetros de capacidade de adsorção e constante de afinidade foram calculados aplicando o modelo de adsorção de Langmuir.

Os resultados são apresentados em três grupos de acordo à trajetória da isoterma de adsorção. O primeiro grupo consta dos análogos que apresentaram a primeira organização das moléculas de peptídeo em concentrações baixas, seguido de um rápido incremento da adsorção a altas concentrações. O segundo grupo de análogos tem um padrão de adsorção que mostra a formação de uma primeira camada a concentrações baixas de peptídeo e uma segunda camada a concentrações maiores. O terceiro grupo contém os análogos cuja adsorção mostra a formação de duas camadas simples e é diferenciado do segundo grupo em que depois da segunda camada, a quantidade adsorbida cresce notavelmente com o aumento da concentração.

Os resultados revelaram um padrão de adsorção mais complexo que a for-

mação de monocamada ou bicamada. Este trabalho constitui a primeira aproximação ao estabelecimento de um modelo de estrutura da camada adsorvida num sistema peptídico.

Palavras-chave: Adsorção, hidróxido de alumínio, peptídeos, vacina, *Plasmodium falciparum*.

INTRODUCTION

In the formulation of vaccines is necessary adsorbing the antigen onto an immunologic adjuvant, like Aluminium Hydroxide gel (AH), capable of amplifying and directing the host immune response against the antigen. For this reason, it was necessary to characterize the adsorption process.

Few studies have been made of peptide adsorption; the most related work has been done with whole proteins because the conventional vaccines contain proteins as antigens. These studies have shown that these molecules become adsorbed on hydrophilic surfaces, mainly by electrostatic attraction. Adsorption may also occur when there is no such attraction due to a particular protein's structural arrangements where attractive intra and intermolecular interactions may happen (1-3).

Protein adsorption studies on AH have shown that these molecules are retained according to Langmuir's adsorption model (2-4), which assumes that all adsorption sites are energetically equivalent, no intermolecular interaction occurs in the system, and adsorption is accompanied by monolayer formation.

Langmuir's equation has been used as a semi-quantitative approach for characterizing physicochemical adsorption parameters, such as adsorption capacity and affinity constant. These parameters have been successfully applied to predict the competitive effect with other proteins which should be taken into account when manufacturing multi-component vaccines adsorbed on AH (5-7).

Other studies have shown that intra and intermolecular interactions may occur depending on the protein structure, causing the formation of multiple antigen layers on the adsorbent surface, a situation further favored at high protein concentrations (2, 5-8).

Models for interpreting the characteristics of adsorption isotherms from solutions describe monolayer or bilayer formation; however, such scheme differs from recent proposals suggesting molecule aggregation on the adsorbent surface (9,10).

For this work we synthesized a peptide which has been considered a good candidate for the development of a vaccine against malaria. This (target) peptide (¹E²V³L⁴Y⁵L⁶K⁷P⁸L⁹A¹⁰G¹¹V¹²Y¹³R¹⁴S¹⁵L¹⁶K¹⁷K¹⁸Q¹⁹L²⁰E) belongs to the amino-terminal region of *Plasmodium falciparum* Merozoite Surface Protein MSP-1.

Given that at physiological pH target peptide has isoelectric point 9.2 and AH zero charge point 11, then it may be thought that there is strong electrostatic repulsion with the surface.

In this vein, we synthesized 20 analogues peptides, replacing each of the amino acids in the target sequence by

aspartic acid which is an amino acid negatively charge at pH 7. (Table 1).

It was found that peptide adsorption on AH depends of several molecular interactions and structural arrangements in the adsorbed layer generating complex isotherms. This suggests the formation of several layers on the adsorbent, essentially agreeing with more recent proposals suggesting molecular aggregation on adsorbent surface.

MATERIALS AND METHODS

Peptide synthesis and characterization

Target Peptide and its analogues were obtained by the solid-phase multiple peptide synthesis method proposed by Merrifield (11) and improved by Houghten (12). Crude peptides were purified by RP-HPLC. Peptide purity was verified on an analytical Lichrosorb® C18 column using 0.05% TFA in water (solvent A), 0.05% TFA in ACN (solvent B), and a 0-70% gradient of solvent B for 30 min. Peptide molecular mass was determined in a Bruker MALDI-TOF mass spectrometer.

Adsorption isotherms on AH

For building adsorption isotherms, twelve peptide solutions of concentration between 0.5 to 12 mg/mL (0.2-5 mmol/L) were prepared at constant temperature (273K) in 0.9% sodium chloride at 7 ± 0.1 pH. AH (Alhydrogel® 2%) equivalent to 1.6 mg of Al/mL (13, 14) was added, shaking the mixture for 12 hours at 150 rpm. Peptide concentration, before and after adsorption, was determined in triplicate by spectrophotometry at 570 nm

using bicinchoninic acid (BCA) (15). The adsorbed amount in mmol/mg Al was determined by the difference between these values and was plotted in terms of the initial solution concentration.

RESULTS AND DISCUSSION

Peptide characterization

Chromatographic analysis of target peptide in pure state gave a 23.6 minutes retention time and mass spectrum showed a 2,348.8 Dalton signal corresponding to the expected peptide molecular mass. The Table 1 shows retention time and molecular mass by analogues peptide.

Adsorption study

There were chosen 17 analogues, which had a solubility greater than 10 mmol/L for adsorption studies. The adsorption results are presented in three groups according to adsorption isotherm trajectory.

The group I (Figure 1) consists of analogues whose adsorption has been defined as close to type 2 isotherm, where the first organisation of peptide molecules was presented at low concentrations, followed by a rapid increase in adsorption to high concentrations.

The group II (Figure 2) consists of analogues having an adsorption pattern showing the formation of a first layer at low peptide concentrations and a second layer which formed over the adsorbed molecules at greater concentrations.

The group III (Figure 3) contains analogues whose adsorption involved

Table 1. Sequence, retention time, and molecular mass of target peptide and its analogues.

PEPTIDE	SEQUENCE	RETENTION TIME (min)	MOLECULAR MASS (Dalton)
Target	EVLYLKPLAGVYRSLKKQLE	23.6	2,348.8
Asp-1	DVLYLKPLAGVYRSLKKQLE	19.71	2,332.5
Asp-2	EDLYLKPLAGVYRSLKKQLE	21.34	2,354.7
Asp-3	EVDLYLKPLAGVYRSLKKQLE	20.65	2,344.5
Asp-4	EVLDLKPLAGVYRSLKKQLE	18.67	2,304.2
Asp-5	EVLYDKPLAGVYRSLKKQLE	18.72	2,354.4
Asp-6	EVLYLDPLAGVYRSLKKQLE	20.74	2,339.5
Asp-7	EVLYLKDLAGVYRSLKKQLE	20.70	2,372.4
Asp-8	EVLYLKPDAGVYRSLKKQLE	17.10	2,355.4
Asp-9	EVLYLKPLDGVYRSLKKQLE	17.56	2,390.5
Asp-10	EVLYLKPLADVYRSLKKQLE	20.72	2,410.3
Asp-11	EVLYLKPLAGDYRSLKKQLE	18.23	2,361.5
Asp-12	EVLYLKPLAGVDRSLKKQLE	18.54	2,296.7
Asp-13	EVLYLKPLAGVYDSLKKQLE	20.98	2,302.4
Asp-14	EVLYLKPLAGVYRDLKKQLE	20.32	2,384.6
Asp-15	EVLYLKPLAGVYRSDKKQLE	18.76	2,345.4
Asp-16	EVLYLKPLAGVYRSLDKQLE	19.72	2,329.5
Asp-17	EVLYLKPLAGVYRSLKDQLE	19.20	2,329.2
Asp-18	EVLYLKPLAGVYRSLKKDLE	19.08	2,332.1
Asp-19	EVLYLKPLAGVYRSLKKQDE	19.72	2,345.8
Asp-20	EVLYLKPLAGVYRSLKKQLD	19.23	2,342.0

the formation of two simple layers, this being differentiated from the group II in that after the second layer had been completed, the amount adsorbed grew notably with increased concentration. The target peptide belongs to this group.

Related studies have shown that the models proposed for interpreting adsorption isotherm characteristics from the solutions describe monolayer or simple bilayer formation; however, our results revealed a more complex pattern.

Probably the initial saturation of the surface was produced by monolayer formation. Then, it occurred a second arrangement of molecules peptide o double layer onto molecules adsorbed. If such interpretation is correct, the isotherm could be separated into two independent concentration zones to apply Langmuir's model. Adsorption capacity and coefficient parameters were calculated by applying the Langmuir's adsorption model (Equation 1).

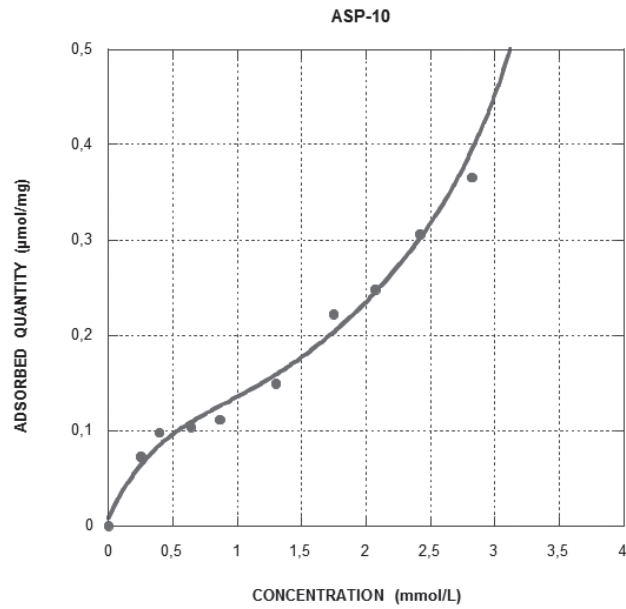


Figure 1. Adsorption isotherm by Group I peptide analogues.

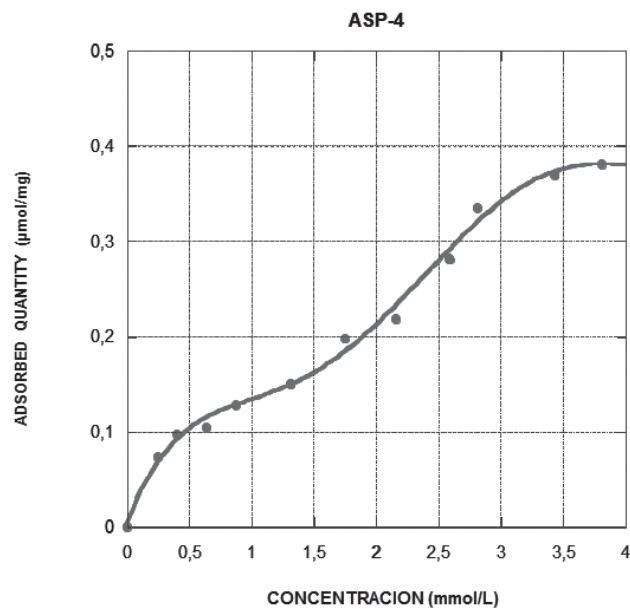


Figure 2. Adsorption isotherm by Group II peptide analogues.

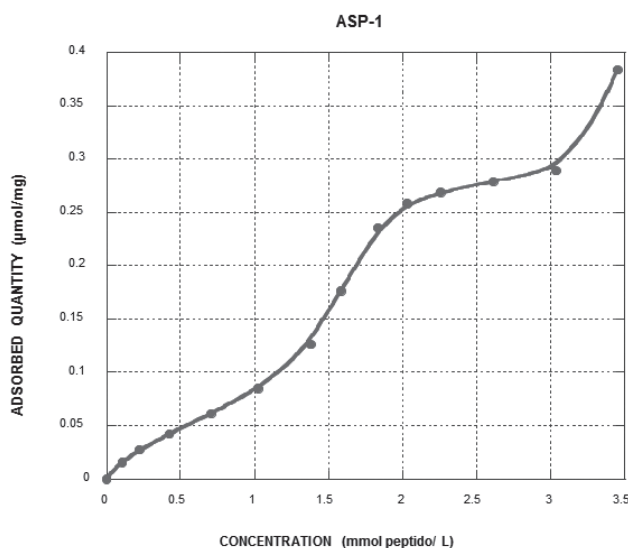


Figure 3. Adsorption isotherm by Group III peptide analogues.

$$\frac{1}{m} = \frac{1}{bCm_n} + \frac{1}{m_n} \quad \text{Equation 1}$$

In Equation 1, m is the adsorbed amount of peptide ($\mu\text{mol/mg Al}$), b is the affinity constant L/mmol , C is the peptide concentration (mmol/L), and m_n is the adsorption capacity ($\mu\text{mol/mg of Al}$).

The Table 2 shows the m_n and b values applying the Langmuir's model in two independent concentration zones, corresponding to the first and the second layer. If adsorbed molecules in the first organisation are found in condensate state on the solid surface, then it is evident that the amount of retained peptide m_n in the second organisation is higher, since adsorbed molecules in this concentration range come into contact with their own condensed phase, which would act itself as dissolvent.

It's interesting to note that when Arginine (R) in Asp-13 peptide (which is an amino acid positively charged) was replaced by Aspartic acid (D), a better adsorption was obtained onto AH. This behaviour was expected due to the decrease of electrostatic repulsion. However, when Lysine (K) in Asp-6 peptide (which is an amino acid positively charged too) was replaced by D, there was not an increase in the quantity adsorbed.

The Table 2 shows that the affinity constant b in the first layer is higher as it measures direct peptide adsorption on the surface, whereas in the second layer, b represents part of the surface interaction in the second layer, which can transcend adsorbed molecules, as well as intermolecular interaction between adsorbed peptide and that forming the double layer.

Table 2. The m_n and b values applying the Langmuir's model by target peptide and analogues.

GROUP	PEPTIDE	m_n^1 ($\mu\text{mol/mgAl}$)	b^1 (Lmmol^{-1})	m_n^2 ($\mu\text{mol/mg Al}$)	b^2 (Lmmol^{-1})
I	Asp-2	0.159	2.693	--	--
	Asp-7	0.129	2.903	--	--
	Asp-10	0.193	2.000	--	--
	Asp-13	0.266	1.864	--	--
	Asp-14	0.239	2.130	--	--
II	Asp-3	0.138	1.190	0.290	0.407
	Asp-4	0.206	1.960	0.390	0.512
	Asp-5	0.128	1.461	0.433	1.149
	Asp-6	0.068	1.362	0.680	0.448
	Asp-8	0.135	2.142	0.935	0.461
	Asp-11	0.225	1.702	0.514	0.821
	Asp-12	0.204	2.018	0.588	0.463
	Asp-15	0.184	1.535	0.590	0.292
	Asp-19	0.202	1.792	0.469	1.407
III	Target	0.086	2.057	0.359	0.324
	Asp-1	0.108	1.484	0.382	1.031
	Asp-18	0.075	2.745	0.210	0.322
	Asp-20	0.099	1.738	0.264	2.780

¹ in the first layer ² in the second layer

This adsorption behaviour can be explained by the fact that peptides are complex molecules and their surface retention depends on their physical and chemical properties, the aminoacids' position in the sequence and three-dimensional structure.

In conclusion, adsorption peptide on AH is a complex process where the final result depends of several molecular interactions and structural arrangements in the adsorbed layer generating complex isotherms, which suggests the formation of several layers on the adsorbent.

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